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Inflammatory Cytokines in Vascular Dysfunction and Vascular Disease

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Abstract

The vascular inflammatory response involves complex interaction between inflammatory cells (neutrophils, lymphocytes, monocytes, macrophages), endothelial cells (ECs), vascular smooth muscle cells (VSMCs), and extracellular matrix (ECM). Vascular injury is associated with increased expression of adhesion molecules by ECs and recruitment of inflammatory cells, growth factors, and cytokines, with consequent effects on ECs, VSMCs and ECM. Cytokines include tumor necrosis factors, interleukins, lymphokines, monokines, interferons, colony stimulating factors, and transforming growth factors. Cytokines are produced by macrophages, T cells and monocytes, as well as platelets, ECs and VSMCs. Circulating cytokines interact with specific receptors on various cell types and activate JAK-STAT, NF-κB, and Smad signaling pathways leading to an inflammatory response involving cell adhesion, permeability and apoptosis. Cytokines also interact with mitochondria to increasie the production of reactive oxygen species. Cytokine-induced activation of these pathways in ECs modifies the production/activity of vasodilatory mediators such as nitric oxide, prostacyclin, endothelium-derived hyperpolarizing factor, and bradykinin, as well as vasoconstrictive mediators such as endothelin and angiotensin II. Cytokines interact with VSMCs to activate Ca^{2+}, protein kinase C, Rho-Kinase, and MAPK pathways, which promote cell growth and migration, and VSM reactivity. Cytokines also interact with integrins and matrix metalloproteinases (MMPs) and modify ECM composition. Persistent increases in cytokines are associated with vascular dysfunction and vascular disease such as atherosclerosis, abdominal aortic aneurysm, varicose veins and hypertension. Genetic and pharmacological tools to decrease the production of cytokines or to diminish their effects using cytokine antagonists could provide new approaches in the management of inflammatory vascular disease.

Keywords

interleukins; oxidative stress; endothelium; vascular smooth muscle; matrix metalloproteinases; aneurysm; varicose veins; hypertension

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INTRODUCTION

Inflammation is an essential component of the immune response to pathogens and damaged cells. Potent inflammatory stimuli include infectious agents, mechanical factors, oxygen radicals, immune complexes, angiotensin II (AngII), inflammasomes, heat shock proteins (HSP), cellular microparticles, adipokines, platelet products and coagulation factors. While the inflammatory response provides an important defense mechanism to injurious agent, injury to healthy bystander cells at the inflammatory site could also occur. Constituents of inflammation include circulating immune cells, vascular cells, connective tissue cells, and extracellular matrix (ECM). Acute inflammation is generally detected within minutes or days by the presence of neutrophils and fluid protein exudates. In blood vessels, acute inflammation involves vasodilation, increased vascular permeability, and blood stasis. Cytoskeletal changes in endothelial cells (ECs) lead to disruption of EC junctions and increased vascular permeability. A delayed sustained response involves inflammatory factors such as C-reactive protein (CRP), plasminogen activator inhibitor-1 (PAI-1), protease-activated receptor (PAR) signaling, CD40/CD40 ligand interactions and cytokines such as interleukins, tumor necrosis factor-α (TNF-α), and interferon-γ (INF-γ). When ECs undergo inflammatory activation, an increase in the expression of adhesion molecules such as selectins, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) promotes the adherence of the inflammatory cells monocytes, neutrophils, lymphocytes, and macrophages and recruitment of additional cytokines, growth factors and matrix metalloproteinases (MMPs). The delayed inflammatory response leads to ECM deposition, granular tissue formation and connective tissue growth [1,2]. Inflammation is usually terminated when the injurious stimulus is removed and all the mediators are dissipated or inhibited. If vascular inflammation progresses unresolved, it can lead vascular disease. This review will focus on the role of inflammatory cytokines in vascular dysfunction and vascular disease. The review will briefly discuss the various cytokines, their sources, receptors and signaling mechanisms. The effects of cytokines on ECs, VSMCs and ECM will be described. The potential role of cytokines in specific vascular disease such as atherosclerosis, abdominal aortic aneurysm (AAA), varicose veins, hypertension (HTN) and preeclampsia will be discussed. The potential benefits of cytokine antagonists in the management of vascular disease will also be discussed.

Classification of Cytokines

Cytokines are a diverse group of soluble short acting proteins, glycoproteins and peptides produced by various immune cells and vascular cells, and act in picomolar to nanomolar concentrations to activate specific receptors and modulate the functions of many cells and tissues. Some cytokines may be membrane-bound or associated with ECM, and switching between soluble and membrane forms may be an important regulatory event. Different cell types may secrete the same cytokine, and a single cytokine may act on several cell types (pleiotropy) and produce multiple biological activities depending on the cell type, timing, and context [3]. Cytokines are also redundant in their activity, meaning similar functions can be stimulated by different cytokines (cross-talk) [4]. Because of the cytokines' functional overlap, their pathophysiological role may be difficult to assess. Also, cytokines are often produced in a cascade, as one cytokine stimulates its target cells to make additional cytokines. Cytokines can also act synergistically (two or more cytokines acting together) or antagonistically (cytokines causing opposing activities).

Generally, cytokines can be classified into the following categories (Table 1 and Table 2):

- Tumor necrosis factors (TNFs)
- Interleukins (ILs), cytokines made by one leukocyte and acting on other leukocytes.
- Lymphokines, initially thought to be produced exclusively by lymphocytes.

- Monokines, initially thought to be produced exclusively by monocytes.

- Interferons (IFNs), thought to be involved in antiviral responses, and include IFN-α, -β, -γ.

- Colony stimulating factors (CSFs), initially thought to support cell growth in semi-solid media, and include granulocyte G-CSF, monocyte M-CSF, and granulocyte-monocyte GM-CSF.

- Transforming growth factors (TGFs) include TGF-β1, 2, and 3, bone morphogenetic proteins (BMPs), activins and inhibins.

- Chemokines, thought to be involved in chemotaxis. The chemokine superfamily is divided into 4 subfamilies (XC, CC, CXC, and CX_3C) based on the presence of a conserved cysteine residue at the NH_2 terminus, and variable region "X". The XC subfamily includes XCL1 and XCL2, which attract lymphocytes. CC chemokines predominantly recruit mononuclear cells. CXC chemokines are subdivided on the basis of the presence of the sequence glutamic acid-leucine–arginine (ELR motif) near the NH_2 terminal. ELR^+ CXC chemokines are neutrophil chemoattractants with angiogenic properties. ELR^- CXCs are lymphocyte chemoattractants with angiostatic properties. The CX_3C subfamily includes CX_3CL1 (fractalkine). Chemokines may have different names e.g. IL-8 (CXCL8), platelet factor-4 (PF-4/CXCL4), macrophage inflammatory protein (MIP)-1α (CCL3), MIP-1β (CCL4), RANTES (regulated on activation, normal T-expressed and secreted, CCL5), and monocyte chemoattractant proteins (MCP)-1 (CCL2), -2(CCL8), -3, and -4 [5].

- Other proteins.

Depending on their cellular source, cytokines are classified into Type 1 cytokines, produced by Th1 T-helper cells and include IL-2, IL-12, IFN-γ, and TNF-β; and Type 2 cytokines, produced by Th2 T-helper cells and include IL-4, -5, -6, -10, and -13. Th1 cytokines tend to drive cellular inflammatory responses including macrophage activation. The Th2 cytokines play a role in distinct inflammatory processes, particularly in allergy, asthma, and atopic dermatitis, and may inhibit certain forms of autoimmunity [6].

Cytokines may also be classified into proinflammatory and anti-inflammatory cytokines. Proinflammatory cytokines are produced predominantly by activated macrophages, involved in the up-regulation of inflammatory reactions and may act as or mediate the following effects:

- Endogenous pyrogens such as TNF-α, IL-1, and IL-6.

- Upregulation of inflammatory reaction such as TNF-α, IL-1, IL-6, IL-19, and IFN-β.

- Stimulation of acute phase reactants such as TNF-α, IL-1, IL-6, IL-11, IFN-γ, TGF-β.

- Chemoattractants such as IL-8, MIP-1α, MIP-1β, RANTES, PF-4, MCP-1, -2, -3.

- Stimulants of proinflammatory cytokines such as IL-12.

Anti-inflammatory cytokines are involved in the down-regulation of inflammatory reactions, and include IL-4, IL-10, IL-13, IFN-α, and TGF-β. However, a clear-cut classification of cytokines as pro- or anti-inflammatory may be difficult, and the net inflammatory response may be determined not only by the balance between pro- and anti-inflammatory cytokines, but also by the timing of cytokine release, the local environment in which they are released, the presence of synergistic or competing factors, cytokine receptor density, and tissue responsiveness to each cytokine.

Sources of Cytokines

Cytokines are produced in response to inflammatory stimuli mainly by macrophages and Th cells, but are also produced by other inflammatory cells, as well as vascular cells and adipocytes (Table 1 and Table 2).

Macrophages are the main source of cytokines. They produce the proinflammatory cytokines TNF-α, IL-1, -6, -12, -15, -18, and -32, as well as the anti-inflammatory cytokines IL-10 and TGF-β. An autocrine activation loop in macrophages may involve self stimulation by IL-12 and IL-18 to produce IFN-γ. Macrophages also express a number of chemokines such as MCP-1/CCL2, MCP-4/CCL13, and IL-8/CXCL8 [7].

T cells secrete numerous cytokines including IFN-γ and IL-4.

Platelets are a rich source of cytokines, chemokines and growth factors. These factors are packaged in storage α-granules, and released during platelet activation. For example, IL-1β is produced by platelets after activation with thrombin. Platelets also secrete CXC chemokines such as PF4/CXCL4 and CC chemokines such as MIP-1 (CCL3) and RANTES (CCL5) [8].

Vascular wall cells are both a source and a target of cytokines. ECs produce IL-1α and IL-1β, while VSMCs produce TNF-α, IL-1α and IL-1β.

Cytokine Receptors

Cytokines exert their biological effects by binding to specific receptors on the surface of target cells. The cytokine receptor superfamily includes several proteins that share extracellular motifs, but have limited similarity in their cytoplasmic domains.

1. Hematopoietin receptor: The hematopoietin cytokine receptor is the best characterized. They are dimers or trimers with conserved cysteines in their extracellular domains and a conserved Trp-Ser-X-Trp-Ser sequence. Examples are receptors for IL-2 through IL-7 and GM-CSF. They generally have two subunits, one cytokine-specific and one signal transducing. For example, the GM-CSF subfamily has a unique subunit that specifically binds either GM-CSF, IL-3 or IL-5 with low affinity, and a shared β subunit signal transducer that increases cytokine-binding affinity. Cytokine binding promotes dimerization of the α and β subunits, which then associate with cytoplasmic tyrosine kinases to phosphorylate proteins that activate mRNA transcription. GM-CSF and IL-3 act on hematopoietic stem cells and progenitor cells and activate monocytes. GM-CSF, IL-3 and IL-5 can also stimulate eosinophil proliferation and basophil degranulation. All three receptors phosphorylate the same cytoplasmic protein, and the antagonistic GM-CSF and IL-3 activities can be explained by their competition for limited amounts of β subunit. The IL-2R subfamily of receptors for IL-2, IL-4, IL-7, IL-9 and IL-15 each has a unique cytokine-specific α chain, but all have a common signal-transducing γ chain. IL-2 and IL-15 are trimers, and share an IL-2R β chain. Monomeric IL-2R α has low affinity for IL-2, dimeric IL-2R βγ has intermediate affinity, and trimeric IL-2R αβγ binds IL-2 with high affinity. IL-2R α chain is expressed by activated (Tac) but not resting T cells. Resting T cells and NK cells constitutively express low numbers of IL-2R βγ [9].
2. IFN receptors: These receptors have the conserved cysteine residues, but not the Trp-Ser-X-Trp-Ser sequence, and include receptors for IFNα, IFNβ, and IFNγ [10].
3. TNF receptors: These receptors have four extracellular domains, and include receptors for soluble TNFα and TNFβ as well as membrane-bound CD40 and Fas. Two TNF receptors, TNFR1 and TNFR2, have been described. The TNF receptors CD40 and Fas bind cell the surface ligands CD40L and FasL on effector T cells. CD40

is expressed on B cell and macrophage plasma membranes. T cell CD40L binding to B cell CD40 stimulates B cell proliferation and isotype switching. T cell CD40L binding to macrophage CD40 stimulates macrophages to secrete TNFα and become more sensitive to IFNγ. T cell FasL binding to Fas leads to activation of caspase proteases that initiate apoptosis of the cell expressing membrane Fas [11].

4. Chemokine receptors: These receptors have seven transmembrane helices and are coupled with G protein. This family includes receptors for IL-8 (CXXR2), MIP-1, MCP-1 (CCR2), fractalkine/CX_3CR1 and RANTES. Chemokine receptors CCR5 and CXCR4 are used by HIV to preferentially enter macrophages and T cells [12].

Cytokine-induced cell signaling

Although the number and roles of cytokines are diverse, the majority of them share common mechanism of action. Many of the cytokines are produced in an inactive form, then undergo enzyme cleavage to the active form. For example, TNFα is synthesized as a 26-κDA type II transmembrane protein, and subsequently cleaved by a membrane-bound enzyme termed TNFα converting enzyme (TACE). The resultant 17-κDa TNFα monomer assembles as a biologically active homotrimer that initiates cell signaling. Also, TGFβ isoforms (TGFβ1, TGFβ2 and TGFβ3 in mammals) are secreted in latent forms that need to be activated before they can bind to signaling receptors. Cytokines interact with specific receptors in target cells, and cause protein kinases associated with the cytoplasmic domains of cytokine receptors to become active by phosphorylating each other [13]. The intracellular signal transduction pathways of cytokines ultimately activate transcription factors such as signal transducers and activators of transcription (STAT). nuclear factor κB (NF-κB), and sma- and mad-related proteins (Smad), and thereby act as gene-regulatory proteins [7]. The majority of proinflammatory effects are through new protein synthesis via these pathways. For example, these pathways synthesize proteins responsible for leukocyte recruitment and adhesion, and for the propagation of the inflammatory process. The activation of these potent signaling cascades also requires specific fine-tuned mechanisms, and negative feedback regulation of these pathways has been described [14]. Such feedback mechanisms include phosphatase regulation of cytokine signaling, protein inhibitors of STATs (PIAS) and suppressors of cytokine signaling (SOCS) proteins.

JAK-STAT Pathway—Most ILs, CSFs, and IFNs mediate their effects through the Janus kinase (JAK)-STAT pathway. After binding to their receptors, many cytokines induce receptor dimerization and recruitment of members of the JAK family, which in turn cross-phosphorylate each other and the cytoplasmic domains of the receptors on tyrosine residues (JAK activation). This provides docking sites for the latent transcription factors STATs. After docking, these transcription factors become phosphorylated then dimerize before entering the nucleus and initiating transcription of target genes. Among the substrates of the JAKs are one or more of the seven members of STATs. Each STAT family member plays a critical role in the biological functions of specific cytokines [15].

NF-κB Pathway—Pro-inflammatory cytokines such as the IL-1 family (including IL-1 and IL-18) and TNF family activate NF-κB and mitogen-activated protein kinase (MAPK) signaling pathways. NF-κB plays a central role in the development of inflammation through further regulation of genes encoding not only pro-inflammatory cytokines, but also adhesion molecules such as E-selectin, VCAM-1 and ICAM-1, chemokines, growth factors, and inducible enzymes such as cyclooxygenase-2 (COX2) and inducible nitric oxide synthase (iNOS). NF-κB is also required for cytokine upregulation of MMP-1, -3 and -9 in human and rabbit VSMCs, and NF-κB inhibition may promote plaque stabilization [16].

Smad Pathway—The TGF-β family members activate the Smad signaling pathway. Members of the TGF family of ligands mediate their effects by binding specific transmembrane type I and type II Ser/Thr kinase receptors. In most cells, TGF signals via TGFβR2 and ALK5 (TGFβR1). The accessory receptors betaglycan and endoglin can modulate signaling via the type II and type I receptors. Betaglycan enhances TGF binding to TGF receptors. Soluble endoglin (sEng) sequesters ligand and thereby inhibits receptor binding [17]. The TGF type I receptors act downstream of type II receptors and determine the signaling specificity within the receptor complex. Upon ligand-induced heteromeric complex formation, the type II receptor transphosphorylates and activates the type I receptor, which subsequently propagates the signal by phosphorylating specific receptor-regulated (R-) SMAD transcription factors at the two C-terminal Ser residues. Activated R-SMADs form heteromeric complexes with a related partner molecule, Co-SMAD (SMAD4 in mammals), and accumulate in the nucleus, where they regulate the expression of target genes such as SERPINE1 (PAI-1) in cooperation with transcription factors, co-activators and co-repressors. In most cell types, TGF induces SMAD2/3 phosphorylation. For example, TGF-β1 induces endothelial barrier dysfunction via Smad2-dependent p38 activation [18]. Inhibitory SMADs, such as SMAD6 and SMAD7 antagonize TGF signalling by inhibiting the activation of R-SMADs [19].

Vascular Effects of Cytokines

The majority of cytokines stimulates immune cell proliferation and differentiation (Table 1 and Table 2). This group includes IL-1, which activates T cells; IL-2, which stimulates proliferation of antigen-activated T and B cells; IL-4, IL-5, and IL-6, which stimulate proliferation and differentiation of B cells; IFNγ, which activates macrophages; and IL-3, IL-7 and GM-CSF, which stimulate hematopoiesis.

Cytokines could also induce vascular cell growth and migration. Both TNF-α and IL-6 induce VEGF expression in cultured A431 human epithelial carcinoma, and L8 rat skeletal myoblast cell lines [20]. IL-1 promotes the growth of rat aorta VSMC via induction of endogenous PDGF production, a process that may participate in the abnormal proliferation of VSMC that occurs early in atherogenesis [21]. Also, IL-1, and inflammatory exudate from zymosan-activated air pouches stimulate chemotaxis of SMCs in an extracellular Ca^{2+}-dependent fashion. These observations suggested that chempattarctants from inflammatory cells such as macrophages and polymorphonuclear leukocytes play a role in the migration of SMC into the intima during atherogenesis, and that Ca^{2+} antagonists might be useful in reducing SMC migration and in treatment of atherosclerosis [22].

Other cytokines such as TNF-α may induce apoptosis. TNF-α initiates apoptosis in many cell types by the recruitment of the DED containing protein caspase-8 to the receptor complex following association of TRADD and FADD to TNFR-1. This receptor recruitment results in autocatalytic activation of caspase-8. Once autocatalytically processed, activated caspase-8 initiates a hierarchical series of caspase activation steps culminating in the activation of effector caspases such as caspase-3 [23]. In EC, transfection of the viral caspase-8 inhibitor CrmA inhibits TNF-induced apoptosis, suggesting that EC activation of caspase-3 involves upstream activation of caspase-8 [24].

Cytokines also promote adhesion of immune cells to ECs and cause an increase in vascular permeability. Studies have examined the effects of TNF-α on the expression of transient receptor potential channel (TRPC) homologues in human vascular ECs and the consequences of TRPC expression on EC permeability. It was found that TNF-α exposure increased TRPC1 expression in human pulmonary artery endothelial cells (HPAEC). Also, thrombin-induced Ca^{2+} influx was twofold greater in TNF-α-stimulated HPAEC than in control cells. When TRPC1 was overexpressed in human dermal microvascular endothelial cell line (HMEC) using TRPC1 cDNA, thrombin-induced depletion of Ca^{2+} stores in these cells caused twofold greater

Ca^{2+} influx than in control cells. Also, inositol 1,4,5-trisphosphate-sensitive store-operated cationic current was increased in TRPC1-transfected compared with control cells. Both thrombin-induced actin-stress fiber formation and decreased transendothelial monolayer electrical resistance were augmented in HMEC overexpressing TRPC1 compared with control cells. TNF-α-induced increased TRPC1 expression in HPAEC also resulted in marked endothelial barrier dysfunction in response to thrombin. These findings indicate that TNF-α induced expression of TRPC1 in ECs is a critical determinant of Ca^{2+} influx and signaling of increased EC permeability [25].

Many of the effects of cytokines on vascular cells could also involve increases in reactive oxygen species (ROS). ROS are generated at sites of inflammation and injury. At low concentrations ROS may function as signaling molecules and participate in the regulation of cell activities such as cell growth. In contrast, at high concentrations, ROS may cause cellular injury and death. ECs are a major target of oxidative stress, which plays a critical role in the pathophysiology of vascular disease. Mitochondria generate ROS as byproducts during ATP production via electron transfer through cytochrome c oxidases. Inflammatory cytokines like TNF-α may increase NAD(P)H oxidase expression/activity and increase ROS. ROS in turn increase chemokine and cytokine expression, which closes vicious circle of inflammation. Several humoral factors may also affect constitutive NAD(P)H oxidase expression in the vascular wall and therefore intracellular ROS production. These factors include AngII, endothelin-1 (ET-1), high glucose or high cholesterol levels [26]. The effect of these factors on baseline ROS production may mediate a major part of their role in inflammation.

Cytokines may have additional specific effects on the vascular ECs, VSM, and ECM. Such effects could affect the mechanism of vascular tone and the signaling pathways of vasoconstriction and vasodilation, vascular cell growth and proliferation, and could also lead to structural changes in the vessel wall architecture and ECM.

Effect of Cytokines on Endothelial cells

ECs are major determinant of vascular tone, leukocyte adhesion, and SMC proliferation. Under normal conditions, the endothelium maintains a vasodilator, antithrombotic and anti-inflammatory state. ECs are major targets of cytokine signals from various immune cells and vascular cells, and could mediate EC dysfunction and vascular inflammation. EC dysfunction, as determined by vasomotor dysfunction, may occur well before the structural manifestation of overt vascular disease such as atherosclerosis, and thus may represent an independent predictor of potential cardiovascular events. ECs play a crucial role in the circulation through the synthesis of vasodilators such as nitric oxide (NO), prostacyclin (PGI_2), and endothelium derived hyperpolarizing factor (EDHF), and vasoconstrictors such as ET-1, thromboxane, and AngII. During EC dysfunction, the synthesis/bioactivity of vasodilators is reduced and the balance tips in favor of the endothelium-derived vasoconstrictors.

Nitric Oxide (NO)—NO is produced during the NOS-mediated conversion of L-arginine to L-citrulline. Two isoforms of NOS are relevant to inflammatory effects, endothelial NOS (eNOS) and inducible NOS (iNOS). Endothelial eNOS is Ca^{2+}-dependent and produces small quantities of NO. Inducible iNOS is usually undetectable in healthy vascular tissue, but is expressed by leukocytes and VSMCs in response to inflammatory stimuli and cytokines. iNOS produces large amount of NO without the requirement for elevated cytosolic Ca^{2+} levels.

Cytokines induce the expression of iNOS, and may induce ECs to produce NO (Vila & Salaices, 2005). For example, TNF-α in combination with IFN-γ from activated Th-1 cells is a potent stimulus of iNOS in ECs [14]. However, TNF-α treatment of rat mesenteric arteries results in inhibition of NO-dependent relaxation [27]. Also, TNF-α inhibits NO-mediated endothelium-dependent relaxation in small coronary arteries via sphingomyelinase activation and

consequent O2•− production in ECs [28]. High concentrations of TNF-α also directly decrease the levels of eNOS mRNA in human ECs [29] and to promote the production of oxygen-derived free radicals by neutrophils, VSMCs and ECs [24]. ROS in turn reduce the bioavailability of NO, and lead to reduction in vascular relaxation.

PGI_2—PGI_2 is an anti-platelet aggregator and a vasodilator with beneficial effects in the circulation. PGI_2 is produced from the metabolism of arachidonic acid by the cyclooxygenase (COX)-1 and COX-2. PGI_2 production is increased during inflammation. Studies have shown that LPS, which stimulates cytokine release, could affect PGI_2 production. Injection of LPS (10 mg/kg) into male Sprague-Dawley rats caused an increase in plasma PGI_2 levels, which was paralleled by a decrease in blood pressure (BP) and an increase in heart rate. LPS injection also increased the mRNA expression of the PGI_2 receptor (IP) in the heart, aorta, and kidney. Co-treatment with the IP antagonist CAY-10441 moderated the LPS-induced changes in BP, heart rate, cardiac output, and vascular resistance. Also, the development of cardiovascular failure was ameliorated by CAY-10441 in spite of the typical LPS-induced increases in plasma levels of cytokines and NO. In vitro, cytokines induced IP expression in rat VSMCs. Incubation of VSMCs with the stable IP agonist iloprost in the presence of the phosphodiesterase inhibitor 3-isobutyl-1-mehylxanthine resulted in higher cAMP levels in cytokine-treated compared with nontreated cells. These observations suggested that the PGI_2/IP system may play a role in LPS-induced cardiovascular dysfunction [30].

Cytokines induce the expression of COX-2 [31], and COX-1 may also be induced at sites of inflammation. Non steroidal anti-inflammatory drugs are the most commonly used remedies for inflammatory rheumatic diseases, and their potential benefit in cardiovascular disease is being recognized.

EDHF—Cytokines may also affect the release of EDHF. Studies have shown that in omental arteries obtained from pregnant women undergoing Cesarean section and examined using isometric wire myography, incubation with TNF-α (1nM) alone did not alter bradykinin-mediated endothelium-dependent relaxation. However, in vessels constricted with a high KCl solution, which inhibits vasodilatation via endothelial-derived hyperpolarising factor (EDHF), TNF-α incubation attenuated bradykinin-induced vasodilatation. Also, TNF-α did attenuate NO and PGI_2-independent endothelial-mediated relaxation. These observations suggested that the vasorelaxant capacity of human systemic arteries is compromised by TNF-α incubation due to inhibition of not only NO/PGI_2 but also EDHF [32].

Angiotensin II—The renin-angiotensin system (RAS) has important modulatory activities in the atherogenic process. Studies have shown that AngII has proinflammatory actions in the vascular wall, and induces the production of ROS, and gene expression of inflammatory cytokines and adhesion molecules. The AngII-induced effects on gene expression are mediated, at least in part, through the cytoplasmic NF-κB transcription factor. Through these actions, AngII augments vascular inflammation, induces EC dysfunction, and, in so doing, enhances the atherogenic process [33]. TNF-α stimulates AngII production in the female reproductive tract and IL-6 upregulates the expression of AT_1R in VSMCs [34].

Endothelin—ET-1 interacts with ET_AR and $ET_{B2}R$ in VSMCs and promotes vasoconstriction. TNF-α and IL-6 stimulate endothelial ET-1 production [35]. Also, studies suggest an increase in cytokine and ET-1 production with age [36], suggesting a potential interaction between cytokines and ET-1 in age-related vascular disease.

Effects of Cytokines on VSM

VSM cells can be induced by a variety of stimuli to synthesize IL-1, -6, -8 and -11, IFN-γ, GM-CSF, MCPs, basic fibroblast growth factor (bFGF), VEGF, PDGF, macrophage migration inhibitory factors (MMIFs)-1α and -1β, and RANTES. Thrombin a serine protease generated at sites of vascular injury is a potent stimulus for cytokine production such as MCP-1 and IL-6 by human VSMCs. This leads to monocyte chemotaxis at sites of injury and in atherogenesis [37]. Cytokine-stimulated HVSMCs release undetectable levels of GM-CSF and G-CSF which inhibits neutrophil apoptosis thus propogating inflammation. IL-1β stimulates the release of both cytokines, with the levels of G-CSF released 10- to 50-fold higher than GM-CSF [38].

SMC migration occurs during vascular injury and atherogenesis and is a necessary process in vessel wall remodeling. During atherogenesis, SMCs migrate to the intima, either from the media or from the circulation via migration of CD34+ hematopoietic progenitor cells, giving rise to smooth muscle progenitor cells. In animal models of vascular injury, intimal and medial thickening is attributable to SMC proliferation and migration from media to intima. These events are regulated by soluble growth factors/ chemoattractants as well as interactions with ECM. SMC migration can be stimulated by cytokines and growth factors such as IL-1β, IL-6, TGFβ1, TNF-α, thrombin, bFGF, insulin-like growth factor-1 (IGF-1), PDGF, urokinase plasminogen activator (u-PA), AngII, and VEGF. These cyokines and growth factors activate signal transduction cascades that trigger remodeling of the cytoskeleton and change the adhesiveness of the cell to the matrix [39].

Cytokines induce macrophage-type NOS in VSMCs. In contrast to the constitutively expressed endothelial eNOS, SMC NOS is independent of Ca^{2+}-calmodulin. IFN-γ and TNF-α synergistically induce arginine-dependent production of NO in rat aortic VSMCs. The capacity for autocrine NO production in VSMCs could be important both physiologically and pathophysiologically, since VSMCs are the most abundant cells of blood vessels and directly control vascular tone, BP, and regional blood flow. Hyperemia is a characteristic component of inflammatory conditions and is necessary for the delivery of both humoral and cellular components of the immune system to the site of inflammation. TNF-α (produced by macrophages) and TNF-β and IFN-γ (produced by T lymphocytes) are released at inflammatory foci. This causes vasodilation by inducing SMC synthesis of NO, which, in turn, contribute to the hyperemia of inflammation. In atherosclerosis, both IFN-γ and TNF are produced in the plaque, which also contains large amounts of "synthetic" SMCs [40]. Cytokine-induced NO synthesis by SMCs may compensate for the loss of EC function and the attenuated endothelium-derived vascular relaxation and participate in the regulation of vascular tone as well as SMC proliferation [41].

$[Ca^{2+}]_i$—Ca^{2+} is a major determinant of VSM contraction and growth. Studies have examined VSMC isolated by laser-capture microdissection from frozen sections of adjacent regions of arteries affected and not affected by atherosclerosis. It was found that localized inflammation and changes in cytokine expression associated with atherosclerosis may affect alternative splicing of the gene encoding the pore-forming $Ca_v1.2α1$ subunit of the Ca^{2+} channel in the human artery VSMCs. It was concluded that inflammation and cytokine-induced molecular and electrophysiological remodeling of $Ca_v1.2$ Ca^{2+} channels may affect VSMC proliferation and function [42].

Electrophysiology and patch-clamp studies have shown that IL-1β, TNF-α, and LPS enhance Ca^{2+} current through voltage-gated channels in isolated VSMCs of rat tail artery [43]. Laser-scanning confocal imaging revealed that TNF-α increased Ca^{2+} spark and Ca^{2+} wave frequency, but reduced global $[Ca^{2+}]_i$ in VSMCs of intact cerebral arteries. It has been suggested that TNF-α activates NAD(P)H oxidase, resulting in an increase in intracellular H_2O_2 that stimulates Ca^{2+} sparks and transient K_{Ca} currents, leading to VSMC

hyperpolarization, reduction in global $[Ca^{2+}]_i$, and vasodilation [44]. Studies have also shown that treatment of rat aorta with IL-1β for 24 hr caused membrane hyperpolarization due to activation of ATP-sensitive K^+ channels and NO-independent inhibition of VSM contraction [45]. However, studies in vascular segments of pregnant rats have shown that treatment with TNF-α and IL-6 enhances vascular contraction, suggesting potential interaction between cytokines and female sex hormones or other factors that are increased during pregnancy on the mechanisms of vascular contraction [46].

Protein kinase C (PKC)—Members of the PKC family of proteins play important roles in signaling for various growth factors, hormones, and cytokines. PKC isoforms include the classic PKC-α, βI, βII, γ, the novel PKC-δ, η, μ,θ), and the atypical PKC-ζ, ι/λ. PKC subtypes participate in the generation of signals for important cellular processes and mediate diverse and, in some cases, opposing biologic responses.

PKC-ζ is essential for inflammatory cytokines and growth factor-induced upregulation of MMP-1, -3, and -9 in VSMCs, most likely by activating NF-κB. Hypertrophy and proliferation of VSMCs are both important in the development of chronic vascular disease. In normal uninjured arteries, VSMCs exist in a nonproliferating differentiated state, the so-called "contractile" phenotype. The expression of these differentiated characteristics is altered in intimal thickening and in atherosclerotic lesions. This dedifferentiation occurs in response to growth factors and growth-promoting surfaces. The dedifferentiated state is also termed the "secretory" or “synthetic” phenotype. There is an inverse association between PKC-α expression and VSMC differentiation [47].

Rho-Kinase—In human pulmonary microvascular ECs TNF-α-induced activation of JNK involves activation of Rho kinase [48]. TGF-β1-induced endothelial barrier dysfunction also involves Smad2-dependent p38 activation and subsequent RhoA activation [18]. Also, TGF-β-induced pulmonary endothelial cytoskeletal reorganization and permeability are partly mediated via RhoA/Rho-kinase dependent signals [49]. Other studies have shown that MCP-1 (CCL2) induces brain EC hyperpermeability via Rho/Rho-kinase and PKCα signaling pathway interactions [50]. Also, a role of Rho family GTPases has been suggested in TNF-α-mediated microfilament cytoskeletal rearrangement and apoptosis in bovine pulmonary ECs [51].

TNF-α also elicit acute RhoA activation, and TNF-α stimulation of MAP kinase, endothelial permeability, and E-selectin expression are inhibited by both the Rho kinase inhibitor Y-27632 and statins [52,53]. The mechanisms underlying the effects of TNF-α on RhoA are unclear. While there may be no direct link between the molecules in the TNF-α signaling cascade and Rho GEFs or GAPs, TNF-α may activate RhoA secondary to activation of sphingosine kinase, resulting in S1P formation and signaling [54]. This is supported by reports that TNF-α–induced MCP-1 expression in response to oscillatory flow is mediated by S1P in human aortic ECs [54]. Also, IL1-β increases nucleotide exchange on RhoA and induces C3-sensitive actin stress fiber formation in HeLa cells [55].

In human coronary VSMCs inflammatory stimuli such as AngII and IL-1β increase Rho-kinase mRNA and protein expression as well as its function as evaluated by the extent of phosphorylation of the ERM (ezrin/radixin/moesin) substrates of Rho-kinase. The expression of Rho-kinase is inhibited by blockade of PKC and an adenovirus-mediated gene transfer of dominant-active Iκ-B, suggesting an involvement of PKC and NF-κB in the intracellular signal transduction pathway for Rho-kinase expression. Also, coronary vascular medial thickening and perivascular fibrosis induced by long-term administration of AngII is suppressed in NF-$\kappa B^{(-/-)}$ mice with reduced expression and activity of Rho-kinase in vivo. These data indicate that inflammatory stimuli such as AngII and IL-1β upregulate Rho-kinase expression and function via activation of PKC and NF-κB in human coronary VSMCs [56]. Rho/Rho-kinase

pathway also mediates AngII-induced MCP-1 mRNA expression and protein amount in rat VSMCs [57]. Also, TGF-β1-induced PAI-1 expression in VSMCs requires Rho/ROCK signaling. However, some studies have demonstrated that IL1-β treatment decreases Rho activation in VSMCs [58].

MAPK—In VSMCs, TNF-α stimulates TNFR1 and leads to activation of MAPK, which is required for VSMC migration. MAPK is also involved in mitogenic signaling by growth factors in VSMCs. Both VSMC migration and proliferation promote their accumulation in vascular lesions. Therefore, strategies targeting the MAPK pathway as a common signaling step in VSMC migration and proliferation may provide new therapeutic approaches for the treatment and prevention of inflammatory vascular disease [59].

Effects of Cytokines on Extracellular matrix

Cyokines and growth factors activate signal transduction cascades that trigger remodeling of the cytoskeleton and promote cell adhesiveness to ECM [39]. The formation of atherosclerotic lesions involves migration of VSMCs from the media into the intima of the artery and their proliferation. These events are regulated by soluble growth factors/chemoattractants as well as interactions with ECM. Integrin receptors link VSMCs to ECM molecules. IL-1β, TNF-α and IFN-γ upregulate VSMC expression of $\alpha_v\beta_1$ integrin, a fibronectin receptor. This enhances the VSMC ability to migrate toward soluble or anchored fibronectin and to adhere to immobilized fibronectin, and thereby augments VSMC proliferative response to mitogens [60]. Other studies describing the effects of cytokines on ECM synthesis have shown that IFN-γ inhibits collagen production by VSMCs, the principal source of collagen in the arterial wall [61].

Matrix metalloproteinases (MMPs) are a family of at least 28 zinc-dependent proteolytic enzymes that can degrade collagen, elastin, and other components of ECM. MMPs are initially secreted as inactive zymogens (pro-MMPs), requiring activation in the extracellular compartment. Similarly, tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) generate plasmin from the inactive precursor plasminogen. Plasmin, a serine proteinase with little direct elastolytic or collagenolytic activity, can indirectly induce ECM degradation by activating MMPs [62]. Cytokine activation of VSMCs can increase the processing of MMPs from inactive zymogens to the active enzymes. Both the constitutive and the cytokine-induced MMPs can digest all major components of the vascular ECM. Also, since cytokines augment the production of MMPs without appreciably affecting the synthesis of TIMPs, locally secreted cytokines may tip the regional balance of MMP activity in favor of ECM degradation [63].

Studies in human and animal models have demonstrated MMP activity within atherosclerotic plaques. Also, stimulation of human or rabbit VSMCs with IL-1 or TNF-α and the growth factors PDGF-bb, mimicking the *in vivo* environment of the atherosclerotic plaque, promote the VSMC synthesis of MMP-1, -3, and -9, which degrade all components of ECM. Also, IL-13 potently induces MMP-2, -9, -12, -13, and -14. IL-1 and TNF-α do not alter the level of TIMP mRNA or protein, leading to a net excess of MMP production that promote breakdown of the vascular ECM [64]. Also, IL-4 and IL-13 augment u-PA and t-PA expression and release from ECs, VSMCs, and monocyte/macrophages [65]. ECs covering atheroma or in the plaque microvasculature contain MMP-1. In pathological conditions associated with local release of cytokines in the vessel wall, enhanced regional expression of vascular MMPs may contribute to VSMC migration and weakening of matrix that would favor plaque rupture. Inflammatory stimuli such as CD40-CD40 ligand interaction also stimulate MMP production from VSMCs [66].

However, the cytokine-induced regulation of MMP expression may be complex. For example, both Th1 and Th2 cytokines such as IFN-γ and IL-4 can induce or inhibit expression of specific MMPs depending on the experimental conditions. IFN-γ induces MMP-9 from human melanoma cells, but inhibits MMP-9 and MMP-12 production by murine and human macrophages. Also, Th2 cytokines such as IL-4 and IL-10 inhibit MMP-1, -2, and -9 production by human macrophages whereas IL-4 induces MMP-12 expression by murine macrophages [66].

Effect of Vascular Factors on Cytokines

While cytokines may influence the release of vascular factors and thereby vascular function, vascular factors may also affect cytokine production by vascular cells. For example, the effects of proinflammatory vascular response can be regulated by anti-inflammatory cytokines such as TGF-β, IL-10, and IL-1ra [67]. *In vivo*, IL-10 most likely exerts its anti-inflammatory effects on the vascular system through inhibition of leukocyte-EC interactions [68] and inhibition of proinflammatory cytokine and chemokine production by macrophages and lymphocytes [69]. IL-10 blocks NF-κB activity through the suppression of IκB kinase activity, preventing IκBα degradation, and suppression of NF-κB DNA-binding activity [70]. IL-10 also affects signaling through extracellular signal-regulated kinase (ERK) 1 and ERK2 and other MAPK-dependent pathways that are potentially important for chemokine and cytokine induction [71]. IL-10 also destabilizes the mRNA of proinflammatory genes.

Endogenous and exogenous sources of NO can limit EC activation by cytokines. NO can reduce cytokine-induced expression of a number of effector molecules such as VCAM-1, E-selectin and IL-6, and, to a less extent, IL-8 and ICAM-1, characteristic of EC activation. The NO anti-inflammatory effects via decreased MCP-1, VCAM-1, and ICAM-1 expression inhibit leukocyte interaction with ECs in early inflammation. The ability of NO to inhibit the expression of EC-leukocyte adhesion molecules and certain proinflammatory cytokines makes it an important regulator of inflammatory trafficking within the vessel wall. NO inhibition of NF-κB may be one of the mechanisms underlying the inhibitory effects of NO on EC activation. NF-κB plays a major role in transcriptionally inducing the expression of adhesion molecules and inflammatory cytokines such as IL-6 and IL-8. Since the activation of NF-κB by TNF-α is thought to occur, in part, via reactive oxygen species, NO may inhibit NF-κB by scavenging and inactivating $O_2^{-}\bullet$. Studies have shown that NO donors inhibit cytokine-induced VCAM-1 expression and reduce monocyte adhesion to cultured ECs. While endogenous levels of NO may not be sufficient to limit cytokine-induced VCAM-1 expression, several cell types such as macrophages, VSMCs and ECs generate 100-fold higher concentrations of inducible NO at the sites of inflammation. Also, higher concentrations of NO could be encountered by cytokine-activated ECs due to their close proximity to VSMCs and macrophages at the site of inflammation and the potential stabilization of NO by formation of potent adducts. Thus EC dysfunction resulting in reduced level of NO activity can lead not only to an imbalance in vascular tone, favoring acute vasoconstriction, but can also impair an endogenous negative feedback loop that limits VCAM-1 expression, leukocyte adhesion, and atherogenesis [72].

Vast evidence has suggested that intracellular ROS production modulate the release of other mediators of inflammation. This is related mainly to the constitutive expression of NAD(P)H oxidases in various tissues [26]. ROS produced by this family of enzymes can regulate adhesion molecule expression on ECs and inflammatory cells, thus affecting cell recruitment to the sites of inflammation. ROS also increase chemokine and cytokine expression. Intracellular ROS also act as second messengers in inflammatory signal transduction, leading to further enhancement of the inflammatory response. At least part of these effects results from the ability of ROS, in particular H_2O_2, to stimulate MAP-kinases activity which leads to activation of several transcription factors [73].

Studies of the mechanisms by which AngII induces expression of inflammatory genes has shown that AngII-induced signal transduction mechanisms overlap those typical of proinflammatory cytokines such as TNF-α [33]. Specifically, AngII activates NF-κB, a protein that controls networks of chemokine-modulating, growth factor-modulating, translational control, and cell survival genes [33]. NF-κB is a family of highly inducible DNA-binding proteins that is regulated by protein processing and by association with a family of cytoplasmic inhibitors, the IBs. AngII has pleiotropic actions at multiple points in the NF-κB activation pathway in VSMCs [33]. AngII not only induces the translocation of the sequestered cytoplasmic NF-κB complex through targeted proteolysis of the IB inhibitors, but also induces the processing of the DNA binding form, NF-κB1, and proteolysis of the proto-oncogene, c-Rel [33]. NF-κB activation appears to be downstream from the NAD(P)H oxidases, because antioxidant treatment interferes with its activation by AngII [33]. The relevance of the NF-κB signaling pathway to inflammatory molecule expression has been supported by studies showing that inhibition of NF-κB activation blocks AngII-inducible IL-6 [33].

Cytokines and their receptors can also be substrates for MMP action. Many of the membrane-bound cytokines, receptors, and adhesion molecules can be released from the cell surface by the action of a subset of MMPs called convertases or adamalysins. This may be one mechanism for the down-regulation of the cytokine cell surface receptors.

Role of Cytokines in Vascular Disease

The role of inflammation in the initiation and progression of vascular disease is increasingly recognized. Epidemiological studies have found increased vascular risk in association with increased basal levels of cytokines such as IL-6 and TNF-α; cell adhesion molecules such as soluble ICAM-1, P-selectin, and E selectin; and downstream acute-phase reactants such as CRP, fibrinogen, and serum amyloid A [74].

TNF-α is an important cytokine in the injured vasculature, where it may function to regulate the expression of PDGF, vascular endothelial cell growth factor (VEGF), fibroblast growth factor (FGF), adhesion molecules, cytokines, and ECM degrading MMPs as well as directly affecting VSMC growth and migration. Other cytokines such as IL-1β, IL-6, and TGF-β contribute to the pathogenesis of specific vascular disease.

Cytokines and Atherosclerosis

Inflammatory and innate immune mechanisms employing monocytes, innate receptors, and innate cytokines may be involved in atherogenesis. Early atherogenesis is characterized by leukocyte recruitment and expression of pro-inflammatory cytokines [75]. Plasma levels and tissue mRNA expression of cytokines and related proteins, such as CRP, have been examined in patients with atherosclerosis. Also, studies on cytokine-deficient animals have provided direct evidence for a role of cytokines in atherosclerosis. *In vitro* cell culture experiments further support the suggestion that cytokines contribute to atherogenesis. Innate cytokines such as IL-1 or TNF may activate ECs, VSMCs, monocytes/macrophages, lymphocytes (T, B, NK), dendritic cells, and mast cells. These vascular cells can actively contribute to the inflammatory cytokine-dependent response in the vessel wall by production of cytokines or eliciting responses to cytokines, or can be involved in cytokine-mediated interaction with invading cells such as monocytes, T-cells, or mast cells. Activation of these pathways results in accumulation of cells and increased LDL- and ECM-deposition which may facilitate subsequent invasions. Thus, vascular cells contribute to the inflammatory pathways involved in both the development and acceleration of atherosclerosis.

Atherosclerosis is characterized by proliferation and dedifferentiation of VSMCs. Also, MCP-1/CCL2 and its receptor CCR2 are key components of atherosclerosis. Studies in mice

have also shown that leukocyte recruitment and expression of pro-inflammatory Th1 cytokines typically characterize early atherogenesis, and modulation of inflammatory mediators reduces atheroma formation [75]. In atherosclerosis, both IFN-γ and TNF are produced in the plaque, which also contains large amounts of "synthetic" VSMCs [40]. Cytokine-induced NO synthesis by VSMCs may compensate for the loss of EC function and the attenuated endothelium-derived vascular relaxation and participate in the regulation of vascular tone as well as VSMC proliferation [41].

The interaction between CD40 and CD40L is also an integral part of the inflammatory pathway in the vascular system. CD40 ligation on cells of the vascular wall promotes mononuclear cell recruitment and contributes to thrombosis in the setting of atherosclerosis [75]. ROS production by mononuclear cells may be a risk factor for vascular disease and mitochondria-derived ROS may also be involved in the pathogenesis of age-related vascular disease. Evidence suggests that the CD40-CD40L interaction might generate ROS and oxidative stress in vascular cells, and CD40–CD40L-mediated generation of ROS might play a role in modulating atherosclerosis [76].

Cytokines and Abdominal Aortic Aneurysm (AAA)

AAA is a permanent localized aortic dilation to >3 cm diameter. The AAA wall is usually laminated with thrombus. Histological features of AAA include adventitial and medial inflammatory cell infiltration, extensive loss of ECM, elastin fragmentation and degeneration, and VSMC apoptosis and medial attenuation. Interstitial collagen type I and III in the media and adventitia provides tensile strength to the aortic wall. In later stages of AAA, collagen degradation exceeds its synthesis, accompanied by excessive degradation of other ECM proteins, notably elastin, ultimately favoring AAA rupture [77].

AAA is an inflammatory disease characterized by the predominance of Th2 cytokine expression and the paucity of Th1 cytokines, especially IFN-γ [75]. In AAA, inflammatory cells (polymorphonuclear neutrophils, macrophages, and T, B, mast and NK cells) infiltrate through the luminal thrombus and the vascular wall. The infiltrating cells secrete various inflammatory factors including cytokines, leukotrienes, ROS and immunoglobulins. Inflammatory cells in the thrombus also release active proteases such as MMP-9 and u-PA [78].

Th1 and Th2 cytokines could influence the outcome of arterial inflammation. Studies have demonstrated elevated circulating levels of IL-1, IL-6, TNF-α, or IFN-γ in patients with AAA [79]. A recent study demonstrated increased expression of IFN-γ and undetectable Th2 cytokines in tissue extracted from ascending thoracic aortic aneurysms (TAA) [80]. Th2 type cytokines IL-4, IL-5, or IL-10 predominate in human AAA lesions, whereas stenotic atherosclerotic lesions preferentially express Th1 cytokines IL-2 and IFN-γ [75].

AAA exhibit increased local production of collagenases, elastase, and MMPs which degrade collagen and elastin ECM proteins [77]. The levels of MMP-1, -2, -3, -9, -12 and -13 [77], serine proteases such as t-PA and u-PA, neutrophil elastase, as well as cysteine proteases such as cathepsin D, K, L and S are elevated in AAA [75]. These proteinases are produced by macrophages, ECs, VSMCs and fibroblasts. Also, CD40 ligation on inflammatory and vessel wall cells induces MMPs and neutrophil elastase from human ECs and monocyte/macrophages [81].

ROS may also contribute to the pathogenesis of AAA. In human, $O_2^{-}\bullet$ levels increase in AAA lesions compared with tissue from adjacent nonaneurysm aorta [82]. In animal models of AAA, ROS initiate Th2 type autoimmune responses; and antioxidant treatment suppresses vasculitis and IgE production in this model [83].

Cytokines and Varicose Veins

In varicose veins, there are reflux, incompetent valves and vein wall dilation resulting in an increase in venous pressure. Phenotypic modulation of VSMCs, altered ECM metabolism, and angiogenesis are the main mechanisms contributing to the morphological and functional modifications of varicose vein remodelling. Inflammatory cytokines and adhesions molecules, especially TGF-β, IL-6, IL-8 and VCAM-1, may be involved in vein valve insufficiency [84]. Increased expression of bFGF and TGF1 by varicose vein cells may play a pivotal role in the hypertrophy of the venous wall, but the exact mechanisms leading to dilatation are unclear. An increase in vein wall tension may augment the expression/activity of MMPs, which induces degradation of ECM proteins and affects the structural integrity of the vein wall. EC injury also triggers leukocyte infiltration, activation and inflammation, which lead to further vein wall damage and fibrosis leading to progressive venous insufficiency and varicose vein formation.

Monocytes/macrophages migrate into the vein wall and valves of patients with venous insufficiency. Venous stasis causes the release of IL-1β, IL-6 and TNF-α by monocyte/macrophage. Also, luminal venous ECs, and ECs in the vasa vasora of refluxing veins are activated, as indicated by the up-regulation of ICAM-1, IL-1α and TNF-α [85].

Cytokines and Hypertension (HTN)

There is a potential link between vascular inflammation and HTN. Cross sectional studies in hypertensive individuals have shown increased plasma and vascular tissue levels of CRP, cytokines such as TNF-α and IL-6, chemokines such as MCP-1 and plasminogen activator inhibitor-1 (PAI-1), and adhesion molecules such as P-selectin and sICAM-1 [86]. However, whether inflammation causes the structural and functional alterations in the vessel wall and leads to HTN or is just a consequence of HTN is unclear. In rats, AngII infusion for 3 days is associated with increased TNF-α production by renal ECs. AngII also stimulates TNF-α production by monocytes, and the adherence of monocytes to ECs. AngII stimulates IL-6 production in VSMCs, and activates NF-κB which further increases the production of cytokines, chemokines and adhesion molecules [87]. The hypertensive response to acute stress and the HTN associated with AngII infusion are attenuated in IL-6 knockout mice, supporting a role of IL-6 in HTN [88]. The role of IL-6 in AngII-dependent HTN is partly explained by increased sodium and water reabsorption as a result of high AT_1R density in proximal tubules [88]. Additional vascular effects of IL-6 on BP involve upregulating AT_1R and enhancing the effects of AngII on vascular inflammation, oxidative stress, vasoconstriction and HTN [34]. AngII increases ROS which further promote the inflammatory process. Also, AngII-induced cytokine production is inhibited by free radical scavengers, supporting a role of ROS in AngII-induced chemokine production. In HTN, mechanical stress on arterial wall and pro-inflammatory humoral stimuli such as AngII and ET-1 induce oxidative stress, which could cause vascular inflammation [26]. The inflammatory factors stimulate ECM deposition and promote VSMC hypertrophy/hyperplasia. Inflammation in turn increases oxidative stress leading to vicious cycle, chronic vascular inflammation and atherosclerosis. Collectively, in HTN, inflammation is associated with EC dysfunction and vascular remodeling either directly or indirectly through oxidative stress.

Studies have measured TGF-β1 mRNA levels in hypertensive rat models and in patients with HTN. Left ventricular TGF-β1 mRNA levels are not different between spontaneously hypertensive rats (SHR) and normal rats, but are increased in stroke-prone SHR (SHRSP) and post-myocardial infarction (MI) rats. Also, renal cortical TGF-β1 mRNA levels are high in DOCA-salt hypertensive rats. AT_1R antagonism and angiotensin converting enzyme (ACE) inhibition decrease left ventricular and VSM TGF-β1 mRNA levels in SHR and post-MI rats, and renal TGF-β1 mRNA in DOCA-salt hypertensive rats and SHRSP. In essential

hypertensive patients, TGF-β1 mRNA and protein are overexpressed. The Arg(25) polymorphism in the TGF-β1 gene is associated with higher BP. Also, high plasma TGF-β1 levels are found in hypertensive patients with microalbuminuria and left ventricle hypertrophy, and AT_1R antagonism and ACE inhibition reduce plasma TGF-β1 levels. Thus, the TGF-β1 overproduction in HTN can be attributed to various factors such as elevated AngII, increased BP per se, increased fluid shear stress and a differential expression of TGF-β1 linked to DNA polymorphism in the promoter [89]. Cross-sectional associations between high BP and plasma levels of CRP, IL-6, and TNF-α have also been reported, but no prospective evidence of these associations is currently available.

Cytokines and Preeclampsia

Normal pregnancy is associated with significant hemodynamic changes in order to meet the metabolic demands of mother and fetus. In 5–7% of pregnancies, women develop preeclampsia, characterized by proteinuria, increased vascular resistance and HTN. Because of the difficulty to perform mechanistic studies in pregnant women, animal models of HTN in pregnancy have been developed. Studies in pregnant rats and rabbits have suggested that reduction in uteroplacental perfusion pressure (RUPP) and the ensuing placental ischemia/hypoxia may represent initiating events of HTN in preeclampsia. Placental hypoxia/ischemia may result in the release of vasoactive factors such as soluble fms-like tyrosine kinase-1 (sFlt-1, an anti-VEGF), endoglin (sEng, an anti TGF-β), AT_1R autoantibody, and cytokines such as TNF-α and IL-6. These factors cause widespread dysfunction of maternal ECs, manifested as decreased formation of vasodilators such as NO and PGI_2, and increased formation of ET-1 and ROS, and vascular reactivity to AngII. These alterations cause HTN by impairing renal pressure-natriuresis and by increasing peripheral and renal vascular resistance [17,90].

The role of inflammatory cytokines in preeclampsia is supported by the observation that the plasma levels of TNF-α and IL-6 are elevated in preeclamptic women [91]. Experimental studies have also shown that chronic infusion of TNF-α or IL-6 in late pregnant rats to increase their plasma levels a 2–3 fold results in significant elevation in renal vascular resistance and BP [46]. Also, the vascular contraction is greater in TNF-α and IL-6 infused compared with control pregnant rats. Furthermore, endothelium-dependent vascular relaxation is reduced in TNF-α and IL-6 infused compared with control pregnant rats possibly due to inhibition of endothelium-dependent NO-cyclic guanosine monophosphate (cGMP) pathway [46].

Cytokine Antagonists

Cytokine antagonists are useful research tools to study the pathways involved in the inflammatory process, and may become useful in the diagnosis and management of vascular disease. Biologic agents that inhibit the proinflammatory activities include cytokine receptor antagonists, anticytokine monoclonal antibodies, and fusion molecules consisting of soluble cytokine receptors combined with human fusion protein constructs or polyethylene glycol.

Etanercept is a soluble TNF-α receptor decoy that binds circulating TNF-α and renders it inactive. Also, chimeric monoclonal antibodies to TNF-α include infliximab, adalimumab, certolizumab pegol. IL-6 variants such as Sant1, Sant5, and Sant7 are IL-6 receptor superantagonists, that prevent the assembly of functional IL-6 receptors and block IL-6-mediated signaling pathways. Anakinra is an IL-1β receptor antagonist. IL-1Ra is an antagonist of IL-1αand IL-1β while IL-18BP is an IL-18 antagonist. Soluble Endoglin (sEng) is a potent TGF-β antagonist.

Other approaches to inhibit the proinflammatory effects of cytokines are to block the cytokine-induced signaling pathways. Endothelial NF-κB signaling orchestrates proinflammatory gene expression in the arterial wall and promotes the pathogenesis of atherosclerosis. Endothelium-

restricted inhibition of NF-κB activation, achieved by ablation of NEMO/IKKγ or expression of dominant-negative IκBα specifically in ECs, results in reduced atherosclerotic plaque formation in ApoE$^{(-/-)}$ mice fed with a cholesterol-rich diet. Also, inhibition of NF-κB abrogates adhesion molecule induction in ECs and reduces macrophage recruitment to atherosclerotic plaques, and expression of cytokines and chemokines in the aorta [92]. In cultured VSMCs, gliotoxin, an inhibitor of NF-κB, inhibits the nuclear translocation of the p65 subunit of NF-κB in response to inflammatory stimuli. Gliotoxin also inhibits VSMC migration and proliferation in response to PDGF-bb. This is associated with rearrangement of the F-actin and vimentin cytoskeleton. In ECs, gliotoxin inhibits nuclear translocation of p65, cell surface expression of adhesion molecules such as VCAM-1, ICAM-1 and E-selectin, and monocytic cell adhesion to a cytokine-activated EC monolayer. Also, in the rat carotid artery balloon catheter injury model, systemic administration of gliotoxin for 10 days decreases neointimal hyperplasia and luminal stenosis and decreases the expression of proliferating cell nuclear antigen in the vessel wall [93].

Perspective and Clinical Implications

Accumulating evidence suggest that vascular inflammation and inflammatory cytokines play a role in the vascular dysfunction associated with vascular disease. Thorough studies are needed to further identify the specific cytokines and vascular receptors involved. Also, the signaling mechanisms via which cytokines could affect vascular function and vascular disease need to be further examined. Such studies would provide the basis for the use of inflammatory cytokines in the diagnosis of vascular disease. Measurement of the chemokine MCP-1 has been used in the diagnosis of atherosclerosis. Also, plasma concentrations of proinflammatory cytokines and chemokines togheter with CRP can predict the risk of coronary events. IL-18 is considered a predictor of future cardiovascular risk. Also, plasma levels of TNF-α and IL-6 may serve as early markers of AAA, varicose vein, and preeclampsia, although sTNFR1 could be a better predictor than serum TNF-α. In contrast, IL-10, a cytokine with anti-inflammatory effects, may be suppressed in vascular disease, and the ratio of IL-6 and IL-10 may constitute a prognostic profile of patient's atherogenicity.

Understanding the regulation of the inflammatory process would also provide the basis for the use of cytokines antagonist approaches in the prevention/treatment of vascular disease. Such approaches include inhibition of cytokine gene transcription and release, antagonizing the effects of cytokines, or inhibition of the cytokine-mediated signaling mechanisms.

Peroxisome proliferator-activated receptor (PPAR) agonists have emerged as a potential tool to modulate the progression of atherosclerosis by exerting direct antiinflammatory and antiatherogenic actions at the arterial walll. Recent evidence suggests that PPARs exert antiinflammatory activities in vascular and immunological cells such as ECs, VSMCs and monocytes/macrophages. In these cells, PPARs may regulate the gene expression and the transcription of proinflammatory genes such as cytokines, chemokines, EC adhesion molecules and MMPs. Thus, PPARs can affect the events involved in atherogenesis such as monocyte/macrophage and lymphocyte recruitment to the arterial wall and foam cell formation [94]. The interaction between CD40 and CD40L is also an integral part of the inflammatory pathway in the vascular system. Therefore, CD40L inhibition has been suggested as a novel therapeutic approach for the prevention of atherosclerotic plaque destabilization and rupture [95].

AngII is a proinflammatory factor that may play a role in the inflammatory response associated with HTN and athreosclerosis. Antagonists of the rennin-angiotensin system can reduce HTN and prevent atherosclerosis in part by reducing vascular inflammation. Also, the release of cytokines by various immune and vascular cells is Ca^{2+}-dependent, and Ca^{2+} channel blockers could reduce cytokine release. Studies have shown that azelnidipine, a dihydropyridine Ca^{2+} antagonist, attenuates stent-associated neointimal formation in non-human primate [96].

Studies in ApoE$^{(-/-)}$ mice fed a high-cholesterol diet have shown that administration of the L-type Ca^{2+} channel blocker amlodipine inhibits atherosclerotic lesion formation and regresses atherosclerosis, and that these effects are partly due to inhibition of inflammatory response and oxidative stress [97].

Studies have also suggested that Rho-kinase, an effector of the small GTPase Rho, is involved in the pathogenesis of arteriosclerosis and in neointimal formation after stent implantation in porcine coronary arteries. Long-term inhibition of Rho-kinase using fasudil suppresses in-stent neointimal formation by reducing vascular inflammation, macrophage accumulation, collagen deposition, and TGF-β1 expression [98].

Manipulation of cytokine levels through diet or natural herbal supplements is an exciting field to explore. For instance, the flavonoid luteolin could reduce cytokine levels. Also, antagonizing the vascular NF-κB and/or hepatic JAK/STAT pathways may modulate the atherosclerotic process [33].

IL-10 has been considered as an anti-inflammatory factor in organ tansplantation. The protective effect of IL-10 against the development of atherosclerosis could be attributed to inflammatory cell deactivation. Additional mechanisms of vascular protection by endogenous IL-10 include decreased O_2^{-}• production in blood vessels in response to LPS, a mechanism that would reduce the impairment of endothelium-dependent NO-mediated vascular relaxation [67].

Other cytokine antagonists include soluble etanercept which is a TNFR that can act a decoy receptor for circulating TNF-α and monoclonal antibodies to TNF-α such as infliximab, adalimumab, and certolizumab pegol. Soluble Endoglin (sEng) can be used as an TGF-β antagonist. IL-1Ra is an antagonist of IL-1α IL-1α and IL-1β while IL-18BP is an IL-18 antagonist and both have anti-atherogenic properties. Anakinra is also an IL-1β receptor antagonist. Also, specific cytokine knock-out mice have been developed, and the potential effects of genetic manipulation of the expression of cytokines and their receptors in vascular disease is an important area of investigation.

Attempts are also being undertaken to inhibit intracellular ROS production in order to limit inflammatory responses. Decoy peptide, which prevents an association of NAD(P)H oxidase subunits may be effective in inflammation related to atherosclerosis [99]. Antioxidant properties of NO are also important in mediating anti-inflammatory properties of NO. NO inhibitory effects on NAD(P)H oxidase can explain successful application of NO gene transfer to limit the extent of vascular inflammation [100]. Strategies targeting the MAPK pathway as a common signaling step in VSMC migration and proliferation may also provide new therapeutic approaches for the treatment and prevention of vascular disease [59].

We should note that systemic blockade of the bioactivity of proinflammatory cytokines may be accompanied by an increased susceptibility to infection. Localized delivery of cytokines and their antagonists can enhance their specificity and effectiveness. Cytokine-eluting stents are being considered as new drug-delivery devices for angiogenic therapy. Local release of cytokines, such as TGF-β, has been shown to induce collateral growth in an experimental model of peripheral artery disease. Other factors might be useful to stabilize atherosclerotic plaques downstream of the site of stent implantation.

List of Abbreviations

AAA, abdominal aortic aneurysm
AngII, angiotensin II
BP, blood pressure

CSF, colony stimulating factors
CRP, C-reactive protein (CRP)
ECM, extracellular matrix
EC, endothelial cell
EDHF, endothelium-derived hyperpolarizing factor
ET-1, endothelin
HTN, hypertension
IFN, inetrferon
IL, interleukin
MAPK, mitogen-activated protein kinase
MCP, monocyte chemoattractant protein
MMP, matrix metalloproteinase
NO, nitric oxide
NOS, NO synthase
$O_2^{-}\bullet$, superoxide anion
PAI-1, plasminogen activator inhibitor-1
PGI_2, prostacyclin
PKC, protein kinase C
RANTES, regulated on activation, normal T-expressed and secreted
ROS, reactive oxygen species
TGF, transforming growth factor
TNF, Tumor necrosis factor
VSMC, vascular smooth muscle cell

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Table 1

Sources, Receptors, Cell Targets and Functions of Representative Cytokines

Cytokine	Producing Cell / Serum Level	Receptor	Target	Function
GM-CSF	Th cells, macrophages, ECs, lymphocytes <8 pg/mL	GM-CSFRα GM-CSFRβ	Progenitor cells, hematopoietic stem cells, neutrophils, macrophages	Growth and differentiation of monocytes, dendritic cells, granulocytes, macrophages Pro-atherogenic
IFN-α	Leukocytes	IFNαR1 IFNαR2	Various	Viral replication, major histocompatibility complex (MHC I) expression
IFN-β	Fibroblasts	IFNβR	Various	Viral replication MHC I expression
IFN-γ	Macrophages Th1 cells Tc cells B cells Natural Killer (NK) cells VSMCs	IFNγR1 IFNγR2	Various Macrophages, lymphocytes, NK cells, EC, SMC	Viral replication Proinflammatory, promotes Th1 immune responses/secretion of Th1-associated cytokines, inhibits ECM synthesis by SMC
			Macrophages	MHC I expression Pathogen elimination
			Activated B cells	Ig class switch to IgG_{2a}
			Th2 cells	Proliferation
			VSMCs	Migration, Proliferation
			ECM	Remodeling Pro-atherogenic
MIP-1α **MIP-1**β	Macrophages	IL-10R2	Monocytes T cells	Chemotaxis
TGF-β1 TGF-β2 TGF-β3	T cells Monocytes	TGF-βR1 TGF-βR2	Monocytes Macrophages	Chemotaxis IL-1 synthesis
			Activated B cells	IgA synthesis
			Various	Proliferation Anti-atherogenic
TNF-α	Macrophages Mast cells NK cells VSMCs T & B cells. <6 pg/mL	TNFR1 (p55) TNFR2 (p75)	Macrophages Various	Cytokine expression Proinflammatory, fever, neutrophil activation, bone resorption, anticoagulant
			Tumor cells	Cell death, tumor necrosis
			ECs	Activate, increase permeability, stimulate adhesion molecules, Pro-atherogenic
TNF-β	Th1 cells Tc cells Platelets, macrophages, Th3, Treg & B cells, SMC	type I and type II Ser/Thr kinase receptors	Phagocytes	Phagocytosis NO production, Pro-atherogenic
			ECs, VSMCs Various	Angigenesis, anti-inflammatory; profibrotic; promotes wound healing, suppresses Th1 & Th2 immune responses

Table 2

Sources, Receptors, Cell Targets and Function of Interleukins.

Cytokine	Producing Cell / Serum Level	Receptor	Target Cells	Function
IL-1α 30.6 kDa IL-1β 30.7 kDa IL-1δ IL-1ε	Monocytes Macrophages T Cells B cells DC ECs, VSMCs <8 pg/mL	IL-1R type1 IL-1R type2	Th cells	Co-stimulation Proinflammatory
			B cells	Maturation, proliferation
			NK cells	Activation
			Various	Inflammation, acute phase response, fever,
			ECs, VSMCs	EC and SMC activation Migration, proliferation Pro-atherogenic
IL-2 (17.6 kDa)	Activated T cells Th1 cells <50 pg/mL	IL-2Rβ IL-2Rγ	Macrophages, T, B, NK cells	Growth, proliferation, activation, Pro-atherogenic
IL-3 (17 kDa)	T cells NK cells Monocytes B cells Mast cells	IL-3Rα IL-3Rβ	Stem cells hematopoietic progenitor cells	Growth, proliferation, differentiation
			Mast cells	Growth, histamine release Pro-atherogenic
IL-4 (17.4 kDa)	Th2 cells, mast cells <15 pg/mL	IL-4Rα IL-4Rγ	Activated B cells	Proliferation, differentiation IgG_1 and IgE synthesis. Pro-atherogenic
			Macrophages	MHC II expression
			T cells T & B cells, mast cells, macrophages, hematopoietic progenitor cells	Proliferation Proliferation and differentiation of B cells (Ig switching to IgG1 and IgE) and Th2 cells (anti-inflammatory by inhibiting Th1 immune responses); stimulates VCAM-1
IL-5	Th2 cells T cells, mast cells, ECs	IL-5Rα IL-5Rβ IL-3Rβ	B cells	Growth, proliferation and differentiation IgA switching
IL-6 (23.7 kDa)	Monocytes Macrophages Th2 cells ECs, VSMCs Fibroblasts Stromal cells 0.16 –7.25 pg/mL	IL-6Rα IL-6Rβ	Activated B cells	Proliferation, Differentiation into plasma cells, pro-atherogenic
			Plasma cells	Antibody secretion
			Stem cells	Differentiation
			Various	Acute phase response
			VSMCs, EC T cells	Proliferation Pro-atherogenic
IL-7	Marrow stroma Thymus stroma Platelets	IL-7Rα IL-7Rγ	Stem cells Monocytes T & B cells	Differentiation into progenitor B and T cells Proinflammatory
IL-8	Macrophages ECs Monocytes, T cells <15 pg/mL	IL-8R	Neutrophils T cells, monocytes	Chemotaxis, Proinflammatory, promotes leukocyte arrest Pro-atherogenic
IL-9 (15.9 kDa)	Th2 cells	IL-9Rα	T cells, B cells mast cells, eosinophils, neutrophils, and epithelial cells	Anti-atherogenic Promotes proliferation and differentiation of mast cells, stimulates IgE production, inhibits monocyte activation, stimulates TGF-β in monocytes
IL-10 (20.5 kDa)	Macrophages Th2 cells Treg and B cells,	IL-10R-1 IL-10R-2	\Macrophages, T & B cells	Inhibit proinflammatory cytokine production Anti-atherogenic

Cytokine	Producing Cell / Serum Level	Receptor	Target Cells	Function
	monocytes mast cells <8 pg/mL			Anti-inflammatory, inhibits Th1 responses, promotes proliferation and differentiation of regulatory T cells
			B cells	Activation
IL-11 (21.4 kDa)	ECs Macrophages	IL-11Rα	Hematopoietic progenitor cells	Hematopoiesis Anti-atherogenic
IL-12α (24.8 kDa) IL-12β (37kDa)	Macrophages B cells Th1 cells	IL-12Rβ2	Activated Tc cells T cells, macrophages	Differentiation into cytolytic T cell (CTL) Proinflammatory, promotes NK and cytotoxic lymphocyte activity; induces IFN- Pro-atherogenic
			NK cells	Activation
IL-13	Th2 cells	IL-4Rα	B cells	Activation of Ig transcription
IL-14	EC, lymphocytes		B cells	B cell growth factor
IL-15 (18 kDa)	EC, macrophages monocytes	IL-2Rβ	T & B cells NK cells, monocytes	Enhances neutrophil chemokine production, cytoskeletal rearrangements, phagocytosis; delays apoptosis Pro-atherogenic
IL-16	Mast cells, $CD4^+$ and $CD8^+$ cells		CD4+	CD4+ T-cell growth factor; Proinflammatory; enhances lymphocyte chemotaxis, adhesion molecule, and IL-2 receptor and HLA-DR expression
IL-17	Human T cells, mouse thymocytes		Fibroblasts, keratinocytes, epithelial cells ECs	Secretion of IL-6, IL-8, PGE2, MCP-1 and G-CSF, induces ICAM-1 expression, T-cell proliferation Pro-atherogenic
IL-18 (22.3 kDa)	Macrophages, ECs, VSMCs	IL-18Rα IL-18Rβ	ECM T cells; NK cells; myeloid, monocytic, erythroid, and megakaryocytic cells	Remodelling, Proinflammatory, induces IFN-γ and other Th1 cytokines, promotes Th1 development and NK activity Pro-atherogenic