1. Introduction

Cardiovascular disease is the leading cause of death in the western world, with 1 in 5 deaths annually attributed to cardiovascular etiology [1]. Vascular diseases, including coronary heart disease, high blood pressure, and stroke, account for the majority of all cardiovascular diseases. Despite aggressive dietary modification, lipid-lowering medications, and other therapies, atherosclerotic vascular disease continues to account for significant morbidity and mortality in westernized societies and is increasing worldwide due to the adoption of western diet and more sedentary lifestyle. It is a significant medical and socioeconomic problem contributing to mortality of multiple diseases including myocardial infarction, stroke, renal failure, and peripheral vascular disease. It will worsen with an increasing number of patients with comorbidity such as obesity, metabolic syndrome, and Type 2 diabetes mellitus; conditions linked with atherosclerotic vascular disease.
or, in the diabetic patient, LDL is also glycated. Oxidized lipids, including an excess of dietary lipid, are among the earliest initiating factors for development of atherosclerosis. Oxidation of LDL exposes numerous epitopes, and oxLDL trapped in the artery and retained there by extracellular matrix acts as an antigenic, proinflammatory compound [3, 4]. Internalization of LDL results in a series of dramatic events which drive the cellular atherogenic response. For example, modified LDL is considered to be chemotactic, leading to extravasation of inflammatory cells to the site of lipid deposition. It was noted that cholesterol feeding rapidly initiated monocyte adherence to the endothelium, leading Gimbrone and colleagues to propose endothelial dysfunction as a major cellular event in atherogenesis [5, 6]. One explanation for increased EC/leukocyte interaction is that internalization of modified LDL can induce gene expression of a host of inflammatory genes, including cytokines and cell adhesion molecules (CAMs). Much of this is mediated by the transcription factor nuclear factor kappa B (NF-κB), a “master switch” for transactivation of inflammatory genes. NF-κB is a redox-sensitive gene regulatory factor that is activated by modified LDL internalization. In this way, a vicious circle of lipid oxidation, LDL internalization, NF-κB activation, gene expression, and leukocyte extravasation is sustained as long as oxidized lipid is present to drive this process. It is this inflammatory gene expression and leukocyte recruitment and extravasation into the artery which gave rise to the inflammatory hypothesis of atherogenesis proposed by Ross [7] which espouses the view that immune cell adhesion to activated, or “inflamed” endothelium is a key cellular event in development of atherosclerosis. The reader is directed to several excellent reviews which focus on atherosclerosis as a localized vascular inflammatory disease [8–11].

2.1. Monocyte/Macrophage. Circulating monocytes are the precursors of macrophage in all tissues. Blood monocytes migrate from the circulation to tissue in response to signals from that tissue in response to tissue damage and infection, where they function to phagocytose damaged cells, foreign bodies, and toxic molecules, such as oxLDL, among others. Modified LDL is a biologically active molecule and acts as a potent monocyte chemoattractant as well as an inhibitor of resident macrophage motility [4]. Monocytes are the first inflammatory cells to be recognized in the nascent plaque, and thus considered preeminent in the process. Systemic depletion of circulating monocytes by clodronate significantly reduces plaque formation, pointing to the preeminence of monocytes in atherogenesis [12]. During the earliest phases of atherogenesis, blood-derived monocytes...
Figure 1: Anatomy of an atherosclerotic plaque. Hemodynamic forces at vulnerable points are predisposed to atherosclerotic lesions. It is at these vulnerable areas of the vasculature that fatty streaks, the earliest recognizable atherosclerotic lesions, form. These streaks are composed of macrophage which collect in the intima and are present in most individuals, including children. Fatty streaks are subclinical, but are the starting point for intermediate fibrofatty lesions. The intermediate fibrofatty lesion consists of several layers of lipid-engorged macrophage termed foam cells, which are intermixed with activated VSMCs which migrate from the media. Both cell types synthesize cytokines, which recruit more VSMCs from the media and immune cells from the circulation, maintaining a chronic, localized vascular inflammatory environment, leading to lesion growth. The fibrous plaque is an advanced lesion of greatest clinical importance and has an organized structure in which a necrotic core containing macrophage foam cells, dendritic cells, and T lymphocytes in various stages of apoptosis, as well as lipid deposits are covered by a dense cap of fibrous connective tissue consisting of activated smooth muscle cells, collagen, and other matrix components. Outward remodeling is a hallmark of this lesion, which helps maintain lumen diameter, but advanced plaques are the most clinically dangerous because of their vulnerability to rupture. Vulnerable plaques are those characterized by thin fibrous caps, and most thrombi result from fracture of this cap. The tendency of a plaque to rupture is associated with the structural integrity of its cap. The most clinically harmful consequences of the advanced plaque is its proclivity to cause thrombotic occlusions. Thin caps tend to correlate with high levels of IFNγ, which inhibits VSMC proliferation and collagen deposition in the cap, while simultaneously increasing synthesis of matrix degradation enzymes. Cellular constituents of an atherosclerotic lesion. The microenvironment of the atherosclerotic lesion is a dynamic gathering of resident vascular and infiltrating inflammatory cells. These cells include endothelial, vascular smooth muscle, T lymphocyte, monocyte, macrophage, macrophage foam cells, and dendritic cells. Cytokines produced by both resident vascular and infiltrating immune cells generate a complex milieu consisting of multiple cytokines which provides the driving mechanism for continued localized inflammation and progression of the atherosclerotic plaque.

home to the intima and subintima followed by transmigration through the endothelium then extravasate into the intima and hence differentiate into the monocyte-derived macrophage. In the intima they engulf oxLDL through receptor-mediated process resulting in a series of highly regulated, albeit maladaptive cellular events which drive the cellular atherogenic response. Oxidized LDL induces macrophage proinflammatory gene expression, including TNFα, IL-1β, and IL-6 [13, 14] through ligand activation of peroxisome proliferator-activated receptor gamma (PPARγ) [15]. Chemokines recruit additional monocytes; in the absence of chemokines, lesion formation is attenuated [16, 17]. Antigenic modified LDL is internalized by macrophage by scavenger receptors as part of a protective response of these cells to sequester modified LDL from the greater circulation. Macrophage can engulf lipid to the extent that they appear “foamy” under microscopy, and lipid laden macrophages are referred to as foam cells. They are metabolically active and are a major source of chemokines and proliferative and inflammatory cytokines and their receptors, which maintain localized inflammation in the intima by recruitment of normally quiescent VSMC from the media into the intima, and additional monocytes from the circulation into the media and subintima. Under particular conditions, macrophage can emigrate out of the plaque, which is associated with a decrease in plaque size and regression of atherosclerosis, pointing to these cells in not only initiation of plaque but also maintenance and expansion of plaque [18].

2.2. Macrophage Polarization. Macrophages are functionally and phenotypically versatile and demonstrate a high degree of plasticity in response to a wide range of stimuli. Macrophages can be classified into phenotypic subsets based on expression of cell surface molecules, cytokine expression, and effector function. The intraplaque cytokine milieu dictates macrophage differentiation into the M1, (proinflammatory), or M2, (reparative) phenotypes. Differential cytokine production is a key feature of polarized macrophages. The M1, or classically activated macrophage, participates in and
propagates proinflammatory processes, and expresses TNFα, IL-1β, IL-6, IL-12, and other proinflammatory cytokines, and thus is considered to be pro-atherogenic. The M2, or alternatively activated macrophage, produces IL-10 and the cytoprotective protein hemeoxygenase-1 (HO-1) and tends to dampen inflammation [19]. This M2 pathway is induced by IL-10, IL-4, and IL-13. M2 macrophage also secretes EC growth and angiogenic factors such as vascular endothelial growth factor (VEGF) and participates in wound repair and neovascularization and are sometimes referred to as reparative macrophages [19-21]. Both M1 and M2 macrophages are present in human lesions, and macrophage phenotypic polarization correlates with lesion progression [22]. For example, in ApoE−/− mice, M1 macrophage dominated over M2 macrophage with disease progression. In regression studies, the proportion of M1 macrophage to M2 macrophage is decreased [22]. Altogether, M2 macrophage, and factors that influence macrophage polarity to M2 may be considered as antiatherosclerotic.

2.3. Vascular Dendritic Cells. Circulating monocytes enter tissue and differentiate into tissue macrophage or dendritic cells (DCs), which share several surface markers with macrophage, including CD80 and CD86 [9]. It is thought that the increase in DC in arterial wall and lesion is from recruitment of pre-DC from the circulation, likely by homing to a chemokine gradient. DCs undergo a maturation process from a circulating DC (pre-DC), into a cell capable of antigen uptake and presentation to T lymphocytes. There are in turn multiple DC subtypes based on cell surface molecule and cytokine expression [23]. Functional, antigen-presenting DCs have extended, dendritic-like cytoplasmic extensions, and are phenotypically similar to Langerhans cells, with a somewhat characteristic dendritic shape. DC can be found in the medial-adventitial border in normal human arteries, but numbers dramatically increase in the intimal, medial, and adventitial compartments of arteries with atherosclerotic lesions [24]. DCs are located in close proximity to T lymphocytes in shoulder regions of human plaque, further supporting a role for these cells in atherosclerosis [25].

Dendritic cells are strictly defined as antigen presenting cells which present antigen to naïve T lymphocytes. In contrast to macrophage, DCs have a predilection to localization and homing to secondary T-cell-rich lymphoid organs where they interact with T cells [26], and thus are suggested to represent a link between the innate and adaptive immune arms of the immune system [27]. Some studies suggest that oxLDL promotes DC activation and maturation, and local lymph nodes do contain oxLDL-reactive T cells [28]. Considering their role as professional APC, it is hypothesized that DCs engulf and process athero-specific antigens [29]. Once activated, they migrate to local lymph nodes, where they present these antigens and activate naïve T cells, driving their activation. An important study demonstrated that DC function, antigen processing and presentation, is enhanced in the dyslipidemic microenvironment [30], suggesting DC are lipid-sensitive regulators of adaptive immunity.

2.4. T Lymphocytes. In addition to macrophage, human atherosclerotic plaque contains approximately 10% T lymphocytes [31, 32]. Studies using Rag- (recombination activating gene-) deficient mice crossed with atherosclerosis-prone mice demonstrate that a deficiency in lymphocytes results in over a 50% reduction in atherosclerotic plaque [33]. Concordantly, adoptive transfer of CD4+ T cells into ApoE−/−/SCID mice enhances plaque development [34]. Together, these important studies emphasize the importance of adaptive immunity in atherosclerosis and suggest that despite their limited numbers, T cells have a major impact on atherogenesis. The overwhelming majority of T lymphocytes found in human plaque are CD4+ T helper (Th1) cells. These are activated and primed by antigen presentation, possibly from macrophage or DC which have internalized oxLDL particles, as T cells cloned from human plaque recognize oxLDL [35]. Like multiple sclerosis, and rheumatoid arthritis, it has been posited that atherosclerosis is a Th1 disease [31], and Th1 lymphocytes drive cell-mediated immunity and are considered proatherogenic as they secrete potent proinflammatory interleukins such as IL-2, IL-12, and cytokines such as IFNγ. These soluble factors not only promote T-cell maturation to Th2 phenotype, but also act on other cells present in the atherosclerotic lesion such as macrophage, EC, and VSMC to further promote local inflammation.

Atherosclerosis is highly influenced by the Th1/Th2 balance, and the interplay between these two phenotypes determines lesion severity [31, 36]. A tip in the balance of these seemingly opposing forces toward the Th2 expression profile has been proposed to have antiatherosclerotic effects. Th1 and Th2 cells originate from a common precursor cell (Th0), and the local cytokine microenvironment in the lesion is thought to determine lineage outcome. A very small proportion of T lymphocytes in the lesion are Th2-biased, and Th2 cytokines such as IL-4 and IL-10 are far less abundant in human and mouse atherosclerotic lesions [32, 37]. Th2 lymphocytes participate in the humoral arm of immunity act on B cells to stimulate antibody production. They are characterized by synthesis of anti-inflammatory cytokines, including IL-4, IL-10, and IL-13 [31]. Importantly, deletion of STAT6, a transcription factor that drives differentiation to Th2, increases atherosclerosis in resistant mice [38], underscoring the antiatherogenic effects of the Th2 arm of adaptive immunity.

T cells which express the FoxP3 (forkhead/winged helix) transcription factor produce TGFβ upon activation are termed T regulatory (T reg) lymphocytes and are found in human atherosclerotic plaque [39]. Depletion of these cells increases atherosclerosis, and adaptive transfer of T reg cells increases plaque stabilization in atherosclerosis-susceptible mice, suggesting a protective role for this T-cell subset [40]. TGFβ induces synthesis of collagen and other matrix proteins as well as is anti-inflammatory, making this an important factor in plaque stabilization. T regulatory cells are considered potent antiatherogenic, and factors which generate T reg, and the cytokines they elaborate are currently the subject of intense study.
2.5. Endothelial Cells (ECs). The vascular endothelium is structurally distinctive as it consists of a single cell layer of specialized endothelial cells lining the interior of blood vessels. Normally functioning endothelium is the key to vascular homeostasis. The endothelium maintains a non-thrombogenic, nonadherent surface; maintains vascular tone by synthesis of vascular dilatory and constricting molecules; acts as a permeability barrier for exchange of substances into artery wall; regulates lipid modification as it is transported into the artery wall. Perturbation of any one of these normal functions leads to atherogenesis. Because it is positioned at the interface between blood circulation and tissue, ECs are equipped with specialized structures to quickly respond to local inflammation and flow disturbances. We will focus on two major functions of ECs as they relate to atherogenesis.

2.5.1. Leukocyte Adherence. The progressive accumulation of transcytosed lipid within the subendothelium is one of the earliest observable changes in atherogenesis and occurs within minutes of hyperlipidemia [41]. OxLDL activate signaling pathways which often converge on the transcription factor NF-κB, which results in transactivation of multiple proinflammatory genes, particularly chemokines and cell adhesion molecules (CAMs), which are proangiogenic [42]. The endothelium is the gatekeeper of leukocyte extravasation, which is a multi-step process including rolling, attachment, and transmigration of leukocytes through the endothelium linking vessel walls. Coordinated expression of chemokines and adhesion molecules is essential to ensure that the appropriate class of inflammatory cells accumulate at the lesion for the appropriate length of time [43]. The repertoire of adhesion molecules expressed by EC is dependent on the local inflammatory milieu. Dyslipidemia induces expression of selectins and ICAM-1, which initiate recruitment and rolling of leukocytes [44]. Continued stimulation elicits expression of ICAM-1 and VCAM-1, which facilitate monocyte adherence by interaction with very late-activation antigen-4 (VLA-4), the counterreceptor to VCAM-1 on the surface of monocytes. These adhesion molecules are a tether for leukocytes to adhere, but also initiate signal transduction events initiated by CAM perturbation. Several in vivo studies have demonstrated that reduction of selectin or CAM expression on vascular EC reduces leukocyte extravasation and subsequent development of atherosclerosis in these mice [45–48]. Secretion of potent chemoattractants such as IL-8 and MCP-1 generate a chemokine gradient which continues recruitment and directional migration of inflammatory cells through the media of the vessel.

2.5.2. Sheer Stress and Mechanotransduction. Because of its anatomical position as a flow interface, ECs are continuously exposed to blood fluid shear stresses. It has long been recognized that atherosclerotic lesions develop preferentially in areas of disturbed or oscillatory blood flow [49]. Vascular regions which demonstrate disturbed flow are the aortic sinus, bifurcations, and curvatures of the renal, coronary, and carotid arteries, all areas with increased lesion formation. These are regions where blood is likely to move more slowly with some degree of eddying, and perhaps a back and forth movement during the cardiac cycle. In contrast, in regions along nonbranching segments, lesions are rarely found [50]. Because of their membrane expression of PCAM-1 and integrins, one unique feature of endothelial cells is that they can act as mechanosensors and mechanotransducers, and are very sensitive to disturbed blood flow. Recent work has shown that oscillatory shear stress, or other nonlaminar flow patterns have profound, proinflammatory effects on EC at the cytoskeletal and gene expression levels. For example, low or disturbed flow induces expression of numerous atherogenic genes including PDGF, MCP-1, and many CAMs, including ICAM-1 and VCAM-1 [51]. Expression of most of these genes is NF-κB-dependent, which is activated in endothelium in areas of disturbed flow. In contrast, EC in areas of laminar flow demonstrate anti-inflammatory effects, with increased production of nitric oxide (NO), increased activation of Kruppel-like factor-2 (KLF-2). KLF-2 is an anti-inflammatory transcription factor, which can regulate the transcriptional response to changes in flow by regulating expression of numerous genes involved in mechanotransduction. Laminar flow is sufficient to reduce TNFα-stimulated expression of VCAM-1 on EC, demonstrating the importance of laminar flow in maintenance of EC homeostasis [52].

2.6. Vascular Smooth Muscle Cell (VSMC). Most studies focus on the role of EC and inflammatory cells, but VSMCs play an important, and perhaps unappreciated role in atherogenesis. The overwhelming majority of cells in the healthy artery are VSMCs. Smooth muscle cells are present in atherosclerotic plaque at all stages, and migration, proliferation, and synthesis of extracellular matrix by activated VSMC contribute to early formation of lesions through several mechanisms, including secretion of inflammatory cytokines, uptake of oxLDL, and elaboration of chemokines [53]. Smooth muscle cells are somewhat unique in that they are capable of two phenotypes, contractile or synthetic, as illustrated by the “response to injury” hypothesis proposed by Ross and Glomset [54]. In the healthy artery, fully differentiated medial VSMC respond mainly to vasoconstricting or vasodilatory peptides, and are normally quiescent. However, VSMCs respond to local inflammation by expressing numerous genes for cytokines, matrix proteins, and cell proliferation regulatory proteins, as well as exhibiting a dedifferentiated transcriptome and phenotype. Perhaps not surprisingly, oxidized phospholipids induce VSMC phenotypic switch to the synthetic, inflammatory phenotype [55]. Activated VSMCs are capable of synthesizing many proinflammatory immune modulators [53, 56, 57] which promulgate autocrine activation of VSMC and recruit macrophage to the developing lesion. They also synthesize and respond to these soluble factors in an autocrine and juxtacrine fashion and migrate from the media into the intima where they proliferate, and, in the case of atherosclerosis, form a cellular “cap” over the developing lipid core. Through their synthesis of matrix proteins, VSMCs are largely responsible for maintaining
plaque stability, and most discussion of VSMC in the context of atherosclerosis tends to focus on their role in cap patency and vulnerability of advanced plaque. Rupture-prone plaques have thin fibrous caps characterized by relatively few VSMCs. VSMCs synthesize the majority of cap components consisting of collagens, and matrix; therefore, VSMCs critically influence cap integrity and consequently, plaque vulnerability. VSMCs respond to the T lymphocyte cytokine IFNγ by not only decreasing synthesis of matrix, but by increasing synthesis of matrix metalloproteinases (MMPs), which weaken and degrade the fibrous cap [11, 58]. Regions of MMP expression often colocalize with hemorrhaged areas, which are also characterized by very few VSMCs, suggesting that combinations of cytokines in concert with IFNγ can lead to SMC apoptosis, or at least attenuation of VSMC proliferation.

Similar to macrophage, VSMCs possess scavenger receptors for oxLDL including lectin-like receptor (LOX-1) and CD36. VSMC scavenger receptors internalize modified oxLDL as ligand, accumulate large amounts of cholesterol esters, and can become foam cells [53, 59]. These receptors are also increased in the presence of inflammatory cytokines such as IL-1β and TNFα. Many scavenger receptors can activate multiple signal transduction cascades and stimulate VSMC migration and proliferation [53, 60]. These gene expression activation events activate the mitogen-activated protein kinase (MAPK) signaling cascade and are Id3-, NF-κB-, JNK- and STAT-dependent [53, 61]. In this way, VSMC lipid internalization also greatly affects the lipid content and inflammatory milieu of the atherosclerotic plaque. Consequently, one therapeutic option would be regulation of expression or function of these receptors which would not only decrease uptake of oxidized LDL, but also reduce signal transduction and maladaptive gene expression. Importantly, oxLDL is proliferative for cultured VSMC inducing a 10-fold increase in DNA synthesis [62]. In vivo, this proliferative effect may be mediated by oxLDL-induced synthesis of VSMC mitogens bFGF and PDGF from other cells in reactive proximity [62]. oxLDL also stimulates expression of chemokines such as MCP-1, inflammatory cytokines like TNFα, and cell adhesion molecules such as VCAM-1, creating a feed-forward autocrine loop and enabling paracrine activation of other cells. In atherosclerotic lesions, VCAM-1-positive VSMCs have been detected juxtaposed with macrophages, suggesting that activated synthetic SMC retain inflammatory cells. The arterial wall can regulate atherosclerosis susceptibility independent of the immune response [63]. C57B6 mice are more susceptible to diet-induced plaque formation than VSMCs isolated from C3H mice, which are somewhat resistant to plaque formation [64]. Cultured VSMCs isolated from CH3 mice are more responsive to oxLDL-induced expression of cytokines and adhesion molecules compared with VSMC propagated from C57B6 mice. The synthesis of these studies supports the notion of VSMC as a versatile inflammatory and structural support cell and advocates the important role of this cell type at all stages of plaque development and as a potential target of antiatherosclerotic therapy.

3. Cytokines, Cellular Communication, and Atherosclerosis

Vascular inflammation is mediated by numerous cell types which communicate with each other through a series of cytokine-receptor-mediated interactions allowing bidirectional crosstalk between resident vascular cells and inflammatory cells [8]. For effective communication to take place, these disparate cell types evolve a common set of soluble cytokine ligands and specific membrane receptors allowing them to transmit their effects into the cell. Most cytokines initiate a complex and varied repertoire of responses on their target cells and can initiate advance, and potentially, resolve atherogenic inflammation. The microenvironment of the atherosclerotic plaque is a dynamic collection of resident vascular and infiltrating inflammatory cells and their cytokine products. This complex milieu consists of multiple cytokines with redundant, pleiotropic, and opposing effects, and the balance of pro- and anti-inflammatory cytokines often determines plaque severity and stability. Cells present in the lesion must interrogate and respond to this multitude of factors in an appropriate fashion, which together often result in the development and progression of atherosclerosis. Thus, our current understanding of the role of any individual cytokine in this disease should be tempered by the fact that no cytokine exists in a vacuum. Addition of a single cytokine to cultured cells, or genetic manipulation of mice, a one gene at a time cannot accurately reflect the in vivo scenario. As such, it may be more appropriate to think in terms of cytokine networks which are temporally expressed and temporally functional.

3.1. Cytokine Classifications. There are currently six families of cytokines classified by structure as well as function, including interleukins (with several subfamilies based on peptide and receptor homology), interferons (IFNs), tumor necrosis factor family (TNF), chemokines, growth factors, and colony-stimulating factors (CSFs) (Table 1). Other categorization strategies have been utilized, and cytokines can be classified based on structural homology of their receptors, in which almost all of the above families of cytokines can be grouped into class I or class II [65]. We can also distinguish cytokines by the more broad functional categorization of their being "pro-" or "anti"-inflammatory. Several anti-inflammatory cytokines have been investigated in non-atherosclerotic disease models. Considering their anti-inflammatory activity, it is anticipated that these will eventually be classified as antiatherosclerotic. Accordingly, when describing a potential role in atherosclerosis, it may be most useful to categorize cytokines into the broad functional categories of pro- and antiatherogenic, or "undetermined."

3.2. Cytokine Signaling Networks and Function. As they can be expressed by cells which also express their receptors, cytokines often signal in an autocrine, paracrine, or juxtacrine fashion. Most cytokines often act in synergy with other cytokines [66] and can initiate multiple cellular processes, often simultaneously. For example, TGFβ can be
mitogenic, drive development, and influence fibrotic gene expression. They are promiscuous in their use of receptor subunits and frequently share one or more homodimers or heterodimers of receptor subunits with other cytokines. For example, IL-19, IL-20, IL-22, and IL-24 all share receptor subunits, but display distinct cellular effects [67]. The observation that several cytokines are synthesized by resident vascular cells and recognized by immune cells supports the hypothesis that vascular cells can be considered as part of the adaptive immune system.

Cytokine engagement of their equivalent receptor initiates a series of intracellular signaling events including activation of a multitude of kinases and transcription factors [68]. Proinflammatory cytokines most often lead to activation of nuclear factor (NF-κB), a transcription factor which acts as a “master switch” for transcription of numerous genes central for inflammatory responses [69]. These genes include matrix and matrix degrading enzymes, cytokines and their receptors, and adhesion molecules which lead to matrix deposition, fibrinolysis, chemotaxis, and leukocyte-endothelial adhesion; all key processes in atherogenesis. Anti-inflammatory cytokines often dampen the activity of proinflammatory cytokines by a series of negative feed back loops mediated by de novo expression of suppressors of cytokine signaling (SOCS) family of proteins, which bind to the cytoplasmic tail of cytokine receptors and signaling intermediates [70].

### 3.3. Proatherogenic Cytokines

Overall, Th1 interleukins are much more prevalent in human atherosclerotic lesions than the Th2 cytokines [32, 71]. Because atherosclerosis is primarily an inflammatory condition, we will categorize our focused discussion into pro- and anti-inflammatory cytokines, with particular attention given to those cytokines with demonstrated effects on atherosclerosis.

#### 3.3.1. IFNγ

IFNγ is expressed by most cell types present in atherosclerotic plaque including macrophage, T cells, and VSMC in human, and experimental lesions in mice. The majority of T cells present in atherosclerotic plaque are Th1-positive cells, which produce abundant amounts of IFNγ. Macrophage, EC, and VSMC produce IFNγ in response to inflammatory stimuli; since IFNγ is a strong inducer of the Th1 phenotype, this leads to a propagation of localized inflammation. IFNγ signals through the JAK/STAT pathway and also activates the SMAD signaling complex leading to inflammatory cytokine production, and in addition to polarizing T cells to the Th1 phenotype, IFNγ also induces macrophage, EC, and VSMC to express proinflammatory genes (reviewed in [66, 68]). IFNγ-induced ICAM and VCAM-1 expression in EC leads to increased extravasation of circulating T cells, thus increasing the inflammatory burden in atherosclerotic lesions.

Several direct lines of evidence from animal models indicate that IFNγ is a potent proatherogenic cytokine. Systemic intraperitoneal IFNγ administration promoted atherosclerosis in ApoE−/− mice [72]. Concordantly, IFNγR−/−/ApoE−/− double knockout mice display a reduced atherosclerotic burden compared with controls [73]. Similarly, gene transfer of a secreted IFNγ receptor decoy in ApoE−/− mice prevented progression of atherosclerotic plaque [73, 74]. There are various modes of action that point to IFNγ as a powerful pro-atherosclerotic agent. In addition to Th1 polarization, IFNγ can reduce cap VSMC proliferation and collagen deposition, while also inducing MMP expression in VSMC, leading toward plaque instability and susceptibility to rupture [73]. Indeed, increased IFNγ levels are seen in unstable plaque [75]. IFNγ can also increase oxidized lipid uptake and foam cell formation in macrophage and VSMC. IFNγ also mediates the pro-atherosclerotic effects of the proinflammatory cytokine IL-18. Injection of IL-18 increased lesion size in ApoE−/− mice [76]; this is inhibited

| Table 1: Cytokines and their sources. EC: endothelial cell, L: lymphocyte, M: monocyte/macrophage, P: platelet, and SMC: smooth muscle cell. |
|-----------------------------------------------|--|-----------------------------------------------|--|-----------------------------------------------|--|-----------------------------------------------|
| **Proatherogenic** | **Cell source** | **Cytokine family** | **Leukocyte phenotype** | **Detected in plaque** | **Primary signaling** |
| IL-1β | M, L, EC, and SMC | IL-1 | – | + | p38/NF-κB |
| IL-6 | M, L, EC, and SMC | IL-6 | – | + | JAK1/STAT3 |
| IL-8 | M, L, EC, and SMC | IL-8 | – | + | GPCR/NF-κB |
| IL-12 | M, L | IL-6 | Th1 | + | JAK2/STAT3 |
| IL-18 | M | IL-1 | Th1 | + | p38/NF-κB |
| IFNγ | M, L, EC, and SMC | IFN | Th1 | + | JAK1/STAT1,2 |
| TNFα | M, L, and SMC | TNF | – | + | p38/NF-κB |

#### Antiatherogenic

| **IL-10** | M, L | IL-10 | Th2 | + | JAK1/STAT1,3,5 |
| **IL-33** | M, L | IL-1 | Th2 | + | p38/NF-κB |
| **TGFβ** | M, L, SMC, and P | TGF | Th2 | + | SMAD2,3 |

#### Likely antiatherogenic

| **IL-4** | L | IL-4 | Th2 | + | JAK1/STAT5 |
| **IL-19** | M, L, EC, and SMC | IL-10 | Th2 | – | JAK1/STAT3 |
in IFN\textgamma\textbar{}ApoE double knockout mice [74]. Together, IFN\textgamma can influence plaque progression at multiple levels and is a potent pro-atherosclerotic cytokine.

### 3.3.2. TNF\textalpha.

Tumor necrosis factor alpha is a member of the TNF superfamily which on the basis of sequence, functional, and structural similarities, currently contains nineteen distinct cytokines. Canonical TNF\textalpha signaling is mediated by p38 MAPK and NF-\textkappa B. Its induction of other proinflammatory cytokines has earned TNF\textalpha the moniker of "master inflammatory cytokine." TNF\textalpha is a pleiotropic cytokine with potently proinflammatory effects and can induce a cascade of proinflammatory gene expression including IL-1\textbeta, IL-8, MCP-1, ICAM-1, VCAM-1, and MMPs in a variety of cell types including lymphocytes, macrophage, EC, and VSMC. Though primarily colocalizing with monocytes and macrophage, TNF\textalpha immunoreactivity is found in all of these cell types in human and experimental atherosclerotic plaque. Similarly, serum TNF\textalpha levels correlate with atherosclerotic plaque burden assessed by carotid artery IVUS, raising the possibility that this cytokine can be used as a noninvasive biomarker for atherosclerosis development [77]. Consistent with its effects on CAM expression, TNF\textalpha promotes leukocyte/endothelial cell interaction in vivo [78]. Perhaps more clinically relevant, TNF\textalpha is associated with plaque rupture as it stimulated production of several MMPs [79], and more ominously, TNF\textalpha induces expression of tissue factor, a thrombogenic protein which plays a role in plaque rupture [80].

In animal models, TNF\textalpha/ApoE double knockout mice have significantly reduced atherosclerotic plaque compared with controls and reduced inflammatory serum cytokine levels and reduced CAMs expression [81]. In studies on ApoE\textsuperscript{−/−} mice treated with recombinant soluble TNF\textalpha p55 receptor, lesion size was reduced [82]. Studies on TNF\textalpha-receptor-deficient mice surprisingly resulted in increased plaque burden, but likely because of elevated cholesterol levels in these mice. Overall, because of its powerful proinflammatory effects on all cell types present in atherosclerotic lesions, TNF\textalpha is a potent protagonist of atherosclerosis and continues to present an attractive target of interventional therapy.

### 3.3.3. IL-1\textbeta.

The IL-1 family is composed of four proteins which share sequence homology: IL-1\textalpha, IL-1\textbeta, IL1 receptor antagonist, and IL-18 [83]. IL-1\textbeta immunoreactivity is found in monocyte, macrophage, EC, and VSMC in human and experimental atherosclerotic plaque, and IL-1\textbeta is produced by macrophage, monocytes, EC, and VSMC. IL-1\textbeta is induced by other proinflammatory stimuli, including TNF\textalpha, and is strongly proinflammatory as it initiates expression of other inflammatory cytokines in multiple cell types [32, 66]. IL-1\textbeta signals activate p38 MAPK and converge on NF-\textkappa B, thus eliciting expression of cytokines and adhesion molecules, and are mitogenic for VSMC and EC [84]. IL-1\textbeta is instrumental in early lesion formation as it increases leukocyte/EC interactions, thus facilitating extravasation [85]. Because it can induce leukocyte extravasation into the developing lesion and also induce cytokine expression in almost every cell present in the lesion; autocrine and paracrine IL-1\textbeta production not only initiates, but maintains the local inflammatory milieu. Multiple animal models demonstrating direct, pro-atherosclerotic effects are reported for IL-1\textbeta. Infusion of IL-1 receptor decoy reduced fatty-streak area in ApoE\textsuperscript{−/−} mice [86]. Similarly, increased lesion area with marked increase in macrophage infiltrate was observed in IL-1 receptor/ApoE\textsuperscript{−/−} double knockout mice [87]. IL-1\textbeta/ApoE double knockout mice showed a 30% decrease in atherosclerosis [88].

### 3.3.4. IL-6.

IL-6 has potent proinflammatory effects in a variety of cell types. EC, VSMC, and macrophage all synthesize IL-6. It is expressed in human atherosclerotic plaque and increased in serum in patients with CAD and unstable angina and is considered an independent risk factor for coronary artery disease [66]. IL-6 enhances CAM expression in EC and VSMC, contributing to extravasation of leukocytes into the developing atherosclerotic lesion. IL-6 signals through the JAK/STAT family of signal transducers and is mitogenic for VSMC. A direct role for IL-6 in atherogenesis has been established as systemic injection of ApoE\textsuperscript{−/−} with recombinant IL-6 resulted in significantly increased plaque [89]. Unexpectedly, lesion size was increased in IL-6/ApoE double knockout mice [90]. Potential mechanisms for this counterintuitive result was an unanticipated increased expression of IL-10, and increased IL-1\textalpha and TNF\textalpha receptors, which were assumed to neutralize bioavailability of these potent proinflammatory mediators. In summary, while a potent proinflammatory cytokine, IL-6 can have complex and somewhat confounding antiatherosclerotic side effects.

### 3.3.5. IL-8.

IL-8 is a potent chemoattractant for leukocytes and other cell types including VSMC [91]. IL-8 is detected in human atherosclerotic plaque, and serum levels are increased in patients with hypercholesterolemia and unstable angina [92]. IL-8 expression is induced in macrophage by oxLDL and EC and VSMC when challenged with TNF\textalpha and IL-1\textbeta. As a potent chemoattractant, IL-8 is an important pro-atherogenic cytokine as it enhances lesion formation by expediting leukocyte extravasation and EC adhesiveness [93]. IL-8 can contribute to plaque instability by inhibition of TIMP-1 (tissue inhibitor of metalloproteinase) expression. In animal models, IL-8 pro-atherogenic effects are likely mediated primarily by leukocytes, as transplantation of IL-8\textsuperscript{−/−} bone marrow into LDLR\textsuperscript{−/−} recipients results in less atherosclerosis compared to IL-8\textsuperscript{+/+} bone marrow [94].

### 3.3.6. IL-12.

IL-12 (p70) is composed of the p35/p40 heterodimeric complex and plays an important role in lymphocyte Th1 differentiation. IL-12 is detected in human atherosclerotic plaques and is proposed to be proatherosclerotic in humans as it enhances T-cell recruitment to the lesion [95, 96]. IL-12 signals through the JAK/STAT complex and induces Th1-polarized cytokines such as IL-2, IL-18, and IFNy, and other cytokines. IL-12 activates the T-bet transcription factor, leading to IFNy production.
and acts synergistically with IL-18 to polarize the innate immune response to a Th1, proinflammatory phenotype [66]. IL-12 can be induced in monocytes by addition of oxidized LDL, is expressed by lymphocytes, activated macrophage and dendritic cells, and is detected in these cells in atherosclerotic lesions. In animal models, atherosclerotic lesions in ApoE/−/− mice are accelerated by injection of recombinant IL-12 [97]. Concordantly, IL-12/−/−/ApoE/−/− double knockout mice have reduced atherosclerotic lesion formation, and LDLR/−/− mice crossed with a mouse with a mutation in which monocytes cannot secrete IL-12 also have reduced plaque formation [98, 99]. Interestingly, IL-12 is potently antiangiogenic and induces an antiangiogenic program mediated by IFNγ inducible genes [100]. It does not appear that IL-12 has direct effects on resident vascular cells; nevertheless, because of its induction of IFNγ, and its potent Th1-polarizing effects, IL-12 is an attractive target for antiatherosclerotic therapies.

3.3.7. IL-18. IL-18 is potentially proinflammatory which may be expected from a cytokine which is related to IL-1 and signals through a receptor with a high degree of sequence homology to the IL-1β receptor. IL-18 and its receptor are detected in human atherosclerotic plaque, and importantly, IL-18 serum levels are elevated in patients with coronary artery disease (CAD) [101]. IL-18 is produced primarily by macrophage, but the IL-18 receptor is present on macrophage, EC, and to a lesser degree, VSMC, which suggests a role for this cytokine in bidirectional communication between inflammatory and vascular cells. IL-18 signal transduction converges on NF-κB and upregulates IL-6, IL-8, and adhesion molecules on monocytes and EC, supporting its proinflammatory and atherogenic properties [102]. IL-18 has pro-atherosclerotic effects in several animal models. Though not strictly in the Th1 category, IL-18 leans toward a Th1 interleukin because it induces production of IFNγ [76]. Systemic administration of IL-18 significantly increased atherosclerotic plaque in ApoE/−/− mice, and IL-18/ApoE double knockout mice had smaller and more stable lesions compared with control mice [103, 104]. IL-18 has a synergistic relationship with IFNγ, as promotion of atherosclerosis by exogenous addition of IL-18 is reduced in IFNγ/ApoE double knockout mice [103, 104]

3.4. Antiatherogenic Cytokines. Cytokine abundance in atherosclerotic plaque is overwhelmingly Th1-oriented [35, 66]. Since so many more pro-atherogenic cytokines and receptors have been identified and characterized, much greater effort has gone into understanding these cytokines and potential for inhibition of their expression or activity. Far fewer studies have pursued characterization of anti-inflammatory cytokines, but perhaps modulation of anti-inflammatory or protective cytokines holds greater therapeutic potential. This section will present both confirmed and potentially antiatherosclerotic cytokines. IL-10, IL-33, and TGFβ have been experimentally confirmed antiatherogenic cytokines.

3.4.1. IL-10. Interleukin-10 is the archetypal Th2 interleukin and the most studied. IL-10 is a pleiotropic cytokine produced by most immune cells, including macrophage, monocytes, and T and B lymphocytes. IL-10 can be detected in these cells in human atherosclerotic plaque. Expression of IL-10 is associated with decreased apoptosis in the lipid core of the lesion [105]. Multiple approaches demonstrate that IL-10 is considered to be atheroprotective by multiple mechanisms including, Th2 T-cell polarization; inhibition of antigen presentation, inhibition of T-cell proliferation, and attenuation of inflammatory gene expression in multiple cell types [66, 106]. The molecular mechanisms of IL-10 activity are complex. IL-10 signals through the IL-10 heterodimeric receptor and activates the JAK/STAT pathway, principally STAT3. IL-10 blocks TNFα-induced MAPK signaling and NF-κB activation in monocytes, macrophage, EC, and VSMC [37, 107]. In cultured monocytes, it has recently been shown that IL-10 can reduce mRNA stability of inflammatory transcripts which contain A/U-rich regulatory elements (ARE) regulatory elements in their 3′ UTR [107]. In this way, IL-10 can dampen the inflammatory response in immune cells at both the transcriptional and posttranscriptional levels.

EC and VSMC do not express IL-10, but IL-10 does have multiple effects on vascular cells, suggesting unidirectional, paracrine/juxtacrine signaling between resident vascular and inflammatory cells. In cultured EC, IL-10 has no attenuating effect on expression of inflammatory cytokines, but can reduce ICAM-1 and VCAM-1 expression. In VSMC, IL-10 did not reduce IL-1β-induced expression inflammatory cytokines, but did reduce VSMC proliferation in an NF-κB dependent manner [108].

Numerous mouse models utilized by various groups have established IL-10 as potently antiatherogenic. In over expression studies, IL-10 transgenic mice have reduced atherosclerotic plaque burden, and transfer of bone marrow from IL-10 transgenic mice into LDLR/−/− mice reduced atherosclerosis [109]. Systemic adenovirus-mediated IL-10 gene expression reduced atherosclerosis by immune cell deactivation and reduction in inflammatory cell infiltrate in plaque [110]. Interestingly, serum cholesterol is reduced in these mice, which also may contribute to decreased vascular inflammation. IL-10 transgenic mice in the C57/B6 background fed an atherogenic diet including cholate had reduced plaque formation, and IL-10/−/− mice in the same background fed the same diet had increased plaque compared with controls. In each case, IL-10 levels correlated with leukocyte infiltration and production of inflammatory cytokines [109, 111]. Concordantly, atherosclerosis is increased in IL-10/ApoE double knockout mice, and transfer of IL-10−/− BM to LDLR/−/− mice demonstrates that it is leukocyte-derived IL-10 that mediates protective effects by polarizing the T lymphocyte Th2/Th1 ratio toward a more anti-inflammatory phenotype [111]. The synthesis of these studies demonstrates that IL-10 is a potent immune modulator that can reduce atherosclerosis by reduction in expression of inflammatory genes, reduction in leukocyte/EC interactions, and polarization of adaptive immunity to the Th2 phenotype.
3.4.2. IL-33. IL-33 is an IL-1 family member expressed by Th2 cells and is considered a Th2 interleukin because it increases synthesis of IL-4, IL-5, and IL-13, while decreasing IFNγ expression. IL-33 is expressed in normal artery, but is increased in macrophage in human atherosclerotic plaque [112]. IL-33 is expressed in several cell types, and expression by macrophage expression has direct suppressive effects on macrophage function. Mice injected with neutralizing decoy receptor developed increased atherosclerotic plaque and had reduced IL-5 expression and anti-oxLDL antibody [112]. In ApoE−/− mice fed a high-fat diet, IL-33 reduced macrophage accumulation into atherosclerotic plaque, but plaque area in these mice by en face staining was not directly assessed [112]. IL-33 can also diminish macrophage foam cell formation by reducing lipid uptake and also increasing cholesterol efflux in these cells [113]. Proposed mechanisms for these effects are reduced expression of scavenger receptors such as CD36 and increased expression of genes, which promote cholesterol efflux such as ApoE, ABCA-1 [113].

3.4.3. TGFβ. Transforming growth factor β is the prototypical and most studied member of a TGF superfamily which currently includes at least 50 different proteins. TGFβ is recognized by a complex family of receptors [114], which signal through the SMAD family of signal transducers. TGFβ is produced by macrophage, T lymphocytes, EC, and VSMC, and detected in these cells in human atherosclerotic lesions. TGFβ is decreased in serum of patients with severe atherosclerosis (reviewed in [115]). TGFβ is considered to be a beneficial cytokine for atherosclerosis, and its antiatherogenic protection is mediated by several mechanisms.

Initially recognized as a profibrotic, wound-healing cytokine with VSMC as a major target, TGFβ has garnered recent attention as a potent anti-inflammatory and Th2-polarizing cytokine. TGFβ has immunomodulatory effects on T-cell differentiation within atherosclerotic lesions [116]. TGFβ plays an important role in T regulatory cell development as it is a costimulatory factor for FoxP3 expression, and the ability of DC to promote T reg cell differentiation is dependent on TGFβ [117]. TGFβ knockout mice are postnatally lethal, but interestingly, severe leukocyte infiltration was observed in every organ throughout the pup, underscoring the potent immunomodulatory effects of this cytokine [118]. Concordantly, TGFβ heterozygous mice fed an atherogenic diet had increased atherosclerotic plaque, endothelial activation, and leukocyte infiltrate [119].

TGFβ contributes to matrix deposition in lesions; administration of neutralizing antibody or soluble TGFβ receptor to ApoE−/− mice increases atherogenesis in those mice, with lesions displaying decreased collagen content [120]. Further, when TGFβ is depleted by injection of mice with a soluble TGFβ-receptor decoy, lesions with decreased collagen content and concomitant intraplaque rupture were observed, which is pertinent for human disease where plaque stability is paramount [121]. Mice receiving bone marrow from mice expressing a dominant-negative TGFβ receptor driven by a T-cell-specific promoter demonstrated increased plaque, T-cell infiltration, and decreased VSMC and collagen content, suggesting that T cells, rather than resident vascular cells, are the major effector for TGFβ activity [121]. The synthesis of these studies suggest that the pleiotropic activities of TGFβ in matrix deposition, immune cell modulation, and T reg development make it a promising subject of study and an attractive therapeutic for attenuation of not only atherosclerosis, but multiple vascular diseases.

3.4.4. IL-4. IL-4 is a confirmed Th2 interleukin produced by activated T cells which promotes autocrine and paracrine immunodampening effects on macrophage and T lymphocytes [37, 66], and some reviews consider IL-4 to be atheroprotective [122]. IL-4 protein and mRNA are detected in human atherosclerotic plaque. IL-4 signals through STAT5, STAT6, and JAK proteins, and can suppress IL-1β and TNFα synthesis in these cells. While conceptually this should be an antiatherogenic cytokine, experimental data do not fully support this assumption. For example, IL-4 can induce VCAM-1 expression in EC which would presumably expedite leukocyte extravasation and leukocyte retention into the vessel wall. Yet other studies show that IL-4 can inhibit macrophage adhesiveness and VSMC proliferation. IL-4−/− mice do not have increased atherosclerosis, and subcutaneous injection of recombinant IL-4 into ApoE−/− mice does not reduce development of atherosclerotic lesions [123]. Further, lesions were actually reduced in area in IL-4−/−/ApoE−/− double knockout mice, and reconstitution of LDLR−/− mice with IL-4−/− bone marrow also reduces lesion formation. IL-4 may represent an example where a Th2 interleukin may not have a clearly direct role in reduction of atherosclerosis, confirming the complexity of the function of the interleukin as well as of atherosclerosis.

3.4.5. IL-19. IL-19 was identified and cloned by database searching for IL-10 homologues [124, 125]. Consequently, IL-19 was originally classified in an IL-10 subfamily which includes IL-19, IL-22, and IL-24, but signals through different combinations of shared receptor chains complexes from these other family members. IL-19 is considered to be a T2 interleukin because it is able to polarize the maturation of human T cells away from the Th1 to the Th2 phenotypes [126, 127]. IL-19 expression was originally assumed to be leukocyte-specific; however, recent reports demonstrate expression by inflammatory stimuli in EC and VSMC [124, 125, 128, 129]. Adenoviral gene delivery inhibits restenosis in angioplasty injured rat carotid arteries [129]. IL-19 has yet to be detected in human atherosclerotic plaque, but is expressed in inflammatory and resident vascular cells in allografted coronary arteries from human transplant patients [129], suggesting that expression is likely in the inflammatory milieu of the atherosclerotic lesion. Furthermore, recent reports indicate that serum concentrations of IL-19 are increased in patients undergoing coronary artery bypass graft (CABG) surgery with cardiopulmonary bypass [130, 131]. In VSMC, IL-19 activates STAT3, decreases migration, proliferation, and expression of inflammatory transcripts, but in contrast to either IL-10 or IL-20, IL-19 does not inhibit NF-κB activity [132]. Rather, IL-19 decreases inflammatory
mRNA transcript stability by inhibition of the mRNA stability factor HuR, which is known to recognize stability elements on the 3′ UTR of inflammatory transcripts [133]. Also in VSMC, IL-19 induces expression of the cytoprotective and antiatherosclerotic protein heme oxygenase-1 [134]. Together, lymphocyte phenotype modulation, reduction in inflammatory transcript stability, and induction of HO-1 represent multiple novel anti-inflammatory, and potentially antiatherogenic mechanisms for IL-19.

4. What Therapies Hold the Most Promise? What Should Be Points of Emphasis for Future Research?

With the emphasis on lipid-initiated inflammation as the driving factor for atherogenesis, it is perhaps not surprising that several efficacious therapies are aimed at altering the immune response. In this section, we present several approaches for therapeutic potential which hold promising possibilities for intervention.

4.1. Statins Are Anti-Inflammatory. Statins are atheroprotective by two mechanisms. Originally designed to target and reduce elevated cholesterol, statins also confer cardiovascular benefit by directly or indirectly modulating the inflammatory component of atherosclerosis (reviewed in [135]). Statins reduce endogenous cholesterol synthesis by inhibition of HMG-CoA reductase, which is the rate-limiting enzyme of the mevalonate pathway through which cells synthesize cholesterol. However, interference with the mevalonate pathway also prevents the synthesis of isoprenoid intermediates which play an important role in the posttranslational modification of the small GTPases Rho, Rac, and Ras, which act as molecular switches upstream of inflammatory signal transduction pathways. Inhibition of isoprenylation results in inhibition of signal transduction pathways and downstream gene transcription. Statins act on endothelial cells, smooth muscle cells, macrophages, lymphocytes, and hepatocytes. In this way, statins modulate inflammatory gene expression in multiple cell types by diminishing activity of transcription factors which regulate genes involved in a wide range of atherogenic pathways. Inhibition of small GTPase activity also modulates cytoskeletal dynamics, and subsequent cell migration, membrane trafficking, and membrane stability. The beneficial anti-inflammatory “side effects” of statins have reinforced the notion that lipid and inflammation remain preeminent targets for antiatherosclerotic therapy, but also point to the small GTPases and components of signal transduction pathways as potential targets as well.

4.2. Inhibition of NF-κB. Inhibition of inflammatory gene expression is a potent antiatherosclerotic strategy. NF-κB is considered to be a “master switch” of inflammatory gene expression [136]. NF-κB is ubiquitous, and it is expressed and active in all cells present in the atherosclerotic lesion. More specifically, many cellular responses to oxidized lipids and inflammatory stimuli are mediated by NF-κB. One approach to exploit NF-κB as a target has been to regulate its interaction with its endogenous regulatory proteins of the IκB family [137]. Several studies in diseases other than atherosclerosis such as allograft vasculopathy and angioplasty-induced restenosis have exploited this protein as a target for vascular diseases, albeit with limited success. One interesting approach has been the use of oligonucleotide decoy of NF-κB consensus binding sites which has been successful to limit restenosis following balloon angioplasty [138]. Pharmacological inhibitors of NF-κB have also been described in the literature and could be promising antiatherosclerotic compounds. Clearly, with its position as a central regulator of inflammatory and pro-atherogenic gene expression, selective inhibition of this important transcription factor is a promising target of antiatherosclerotic therapy.

4.3. Anticytokine Therapy. Activation of immune and resident vascular cells by atherogenic stimuli is initiated and sustained by a complex network of cytokines. With the preeminent role of cytokines in atherogenesis, they hold obvious promise as targets to combat this disease. Approaches to blockade of bioavailability of pro-atherogenic cytokines include cytokine receptor antagonists, cytokine-specific monoclonal antibodies, and soluble cytokine decoy receptors. Potent proinflammatory cytokines including TNFα, IL-1β, and IL-18 have naturally occurring endogenous inhibitors which could potentially be harnessed as antiatherosclerotic therapy [139]. Use of a TNF receptor protein has shown some efficacy to combat rheumatoid arthritis, but efforts to attenuate atherosclerosis are forthcoming [140]. Considering pleiotropic and redundant roles of many inflammatory cytokines, key issues of selectivity and tissue specificity remain to be addressed.

4.4. Upregulation of Anti-Inflammatory Cytokines. If inhibition of proinflammatory cytokines hold promise for reduction of atherosclerosis, then induction of anti-inflammatory cytokines also need to be considered as a viable therapeutic approach. As discussed, several anti-inflammatory interleukins have potentially antiatherosclerotic effects, including IL-4, IL-10, IL-19, and IL-33. Since overexpression has been proposed to attenuate other inflammatory conditions such as rheumatoid arthritis, colitis, and asthma, they may also prove attractive as an approach for attenuation of atherosclerosis. Several promising studies in atherosclerosis-susceptible mice have implicated IL-10 as a potential antiatherosclerotic therapeutic, but additional studies are necessary for IL-19 and IL-33 [106, 109–111]. Caution needs to be taken as increases in anti-inflammatory cytokines have the potential for untoward side effects of immunosuppression and susceptibility to infection. One approach may be to administer one or more anti-inflammatory cytokines to alter the balance of Th1/Th2 cytokine ratio, thus modulating endogenous adaptive immunity to a more anti-inflammatory profile. Little has been published regarding potential protective effects of Th2 interleukins on resident vascular cells in addition to inflammatory cells. Presently, most studies which focus on Th2 interleukins currently consider them to be
indirectly antiatherogenic by dampening the host immune response, thus reducing inflammation and subsequent lesion formation [37, 111]. However, recently, IL-19 has been shown to reduce angioplasty-induced restenosis in rats, and inflammatory gene expression in human VSMC [129, 132]. IL-19 has also been shown to induce HO-1 expression and reduce ROS in human VSMC [134]. Together with its Th2-inducing properties, IL-19 may represent an attractive therapeutic modality to attenuate atherosclerosis.

4.5. Immunization. Immunization with oxidized LDL induces antibody against oxLDL and significantly reduces experimental atherosclerosis [141, 142]. Immunization with other antigens, such as heat shock proteins or ApoB100 fragments, have also reduced athero in mice, implicating vaccination as a promising approach to prevent or attenuate atherosclerosis [143]. Importantly, immunization with ApoB100 peptide induced antigen-specific T reg generation [144]. The precise mode of action of protection remains to be elucidated. It is speculated that induction of antibody by immunization increases clearance of oxLDL; this is supported by the observation that protection can be correlated with the titer of the antibody [142]. Caution needs to be taken to avoid molecular mimicry and cross-reactivity that could have deleterious autoimmune responses; nevertheless, future studies to determine the most effective antigen and vaccination regimen need to be performed to optimize this promising and novel strategy.

4.6. Stimulation of T Regulatory Cells. Considering the antiatherogenic effect of T regulatory cells in attenuation of atherosclerosis, therapy to induce generation of T reg cells is potentially a powerful approach to attenuate atherosclerosis. Several studies have pursued this approach. In one such study, mice treated with CD3-specific antibody induced CD4+ CD25+ T reg cells [145]. Further, these cells secrete high levels of TGFβ, which dampens the immune response as well as increases plaque stability. In theory at least, any prolonged introduction of a peptide could stimulate T regulatory cells and induce immunological tolerance. This approach has been pursued as a therapy for allergies and arthritis for several years, but has yet to be successfully exploited to treat atherosclerosis.

5. Summary and Conclusions

Atherosclerotic vascular disease remains a significant cause of mortality and socioeconomic burden in the westernized world and will increase worldwide due to the adoption of a western diet and sedentary lifestyle. Atherosclerosis is an enormously complex and dynamic disorder involving multiple cell types and soluble factors. Several types of immune cells as well as resident vascular cells respond to hyperlipidemic environment in a maladaptive fashion resulting in localized vascular inflammation recognizes as atherosclerotic plaque. However, we have learned much in the previous two decades about the etiology, progression, and maintenance of atherosclerosis. Our recent appreciation that inflammation plays a critical role in atherosclerosis has increased our opportunity to devise therapeutic treatment to reduce or prevent atherosclerosis. As an example, since the balance of pro- and anti-inflammatory cytokines dictates plaque content and can determine plaque severity and stability, tipping the balance toward anti-inflammation may have beneficial effects. Despite their complexity, modulation of signaling pathways and transcription factors which regulate expression of those cytokines also offer opportunity for therapeutic intervention. However, many issues concerning etiology, detection, cellular dynamics, and plaque vulnerability remain unresolved. In summary, vascular inflammatory diseases in general and atherosclerosis in particular represent an opportunity and challenge. Our understanding of atherosclerosis at the tissue, cellular, and molecular levels are crucial in our ability to devise potent and specific therapeutic modalities to combat this disease.

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References


