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Therapeutic Implications of Inflammation in Atherosclerotic Cardiovascular Disease

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Atherosclerosis represents a spectrum of pathologic lesions with diverse clinical sequelae. In this review, we build upon the paradigm that arteriosclerosis represents an inflammatory disease. By examining mechanisms involved in the response to vascular injury, we can more effectively implement targeted therapy aimed at halting or regressing arteriosclerosis.

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In 1755, Von Haller [1] coined the term atheroma to describe an arterial plaque with a yellow-colored pustulous core. Since then, the pathologic delineation of the arteriosclerotic lesion has progressed from gross description to the cataloging of genes and intracellular transcription factors. Although our ability to characterize these lesions has evolved, arteriosclerotic cardiovascular disease remains the principle cause of morbidity and mortality in our society. Numerous strategies have tried to promote regression of or to avert arteriosclerosis; few have succeeded in randomized, prospective trials. In this review, we summarize current concepts of arteriogenesis and propose a paradigm that could allow for effective therapy.

What is Atherosclerosis?

Atherosclerosis is a pathologic condition that cannot be defined as a single disease entity. It represents a wide spectrum of lesions with diverse patterns of distribution and clinical sequelae. To eliminate semantic confusion, the American Heart Association recently formulated several consensus statements that create a histologic classification of atherosclerosis (Table 1) [2, 3]. The initial or type I lesions, consisting of lipid deposits in the intima, have been well documented in infants and children. Fatty streaks, or type II lesions, are visible as yellow-colored streaks, patches, or spots on the intimal surface of arteries. Microscopically, these lesions are characterized by intracellular accumulation of lipids (ie, foam cells). Although the sequence of events is not always consistent, fatty streaks then progress to the type III or intermediate lesion. This growth is characterized by extracellular pools of lipid that are generally not clinically perceptible. However, when these pools coalesce to create a core of extracellular lipid (type IV lesion or atheroma), the blood vessel architecture is sufficiently distorted that this type becomes clinically perceptible. With smooth muscle cell proliferation and collagen deposition, the atheroma becomes a fibroatheroma (type V). This lesion is susceptible to surface defects, with subsequent hemorrhage or thrombus (type VI lesion), resulting in vessel occlusion.

Vascular remodeling and its clinical sequelae can occur at an early age. In 1953, Enos and colleagues [4] reported autopsy findings from 300 American male battle casualties (average age 22 years) in Korea. He noted that 77% of the hearts had some gross evidence of coronary arteriosclerosis. Thirty-nine percent had luminal narrowing, estimated to be 10% to 90%, and 3% had plaques causing complete occlusion of one or more coronary vessels. A recent study of 111 trauma victims corroborated that study by identifying a 78% incidence of arteriosclerosis, with left main or significant two- or three-vessel involvement noted in 20% [5]. These studies show that strategies aimed at primary prevention must be initiated at a relatively young age.

Atherogenesis and the Response to Injury

Epidemiologically, traditional cardiovascular risk factors include tobacco use, hyperlipidemia, hypertension, diabetes mellitus, and family history. How is it that these seemingly unrelated risk factors produce similar endpoints? Are there parallel pathways that lead to a final lesion, or do these risk factors activate signals that converge to a few dominant events? Several theories have been offered to explain atherogenesis. The lipid hypothesis suggests that the cellular changes in arteriosclerosis are reactive events in response to lipid infiltration. The influence of lipids on cardiovascular disease is clearly evidenced in patients with genetic hyperlipidemias, in which homozygous individuals rarely live beyond 26 years. Antilipid therapy is one of the few strategies that has induced regression of arteriosclerosis in randomized, prospective clinical trials [6]. However, nearly 20% of those adequately treated patients had adverse cardiovascular events, emphasizing the importance of nonlipid risk factors [7]. Other theories of atherogenesis include the thrombogenic hypothesis (abnormal blood elements acting as a nidus for fibrin deposition), the mesenchymal hypothesis (proliferation of smooth muscle cells and collagen deposition), and the monoclonal hypothesis (lesion development from a few mutated smooth muscle cells) [8–10].

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Table 1. Histopathologic Classification of Atheroclerosis[a]

Type	Lesion	Histopathology
I	Initial	Adaptive intimal thickening at lesion-prone locations; aberrant smooth muscle cells and isolated macrophages
II	Fatty streak	Intracellular accumulation of lipids; macrophages form foam cells
III	Intermediate	Continued accumulation of lipids with small pools of extracellular lipids
IV	Atheroma	Coalescence of extracellular lipid pools into a lipid core with a thin fibrous cap
V	Fibroatheroma	Accelerated proliferation of smooth muscle and collagen synthesis; multiple lipid cores and fibrotic layers, with or without calcification
VI	Complicated	Surface defect with hemorrhage into plaque and subsequent thrombosis

[a] As endorsed by the American Heart Association, Stary and colleagues [3]).

In 1974, Ross and associates [11] postulated that atherogenesis was dependent on cellular interactions in the vessel wall after endothelial injury. Release of growth factors by stimulated monocytes and platelets promoted vascular smooth muscle cell (VSMC) proliferation. The premise that atherogenesis results from an exaggerated response to injury has evolved into an attractive unifying hypothesis of vascular disease and repair (Fig 1). Injury includes more than direct physical trauma, as seen with angioplasty, hypertension, and shear stress (atherosclerotic lesions typically occur at vessel bifurcations). Vessel damage also is initiated by other insults, including viruses, bacteria, nicotine, homocysteine, and oxidized low-density lipoproteins (LDL). Endothelial disruption or dysfunction allows for adhesion and transmigration of circulating monocytes, platelets, and T lymphocytes. Within the developing lesion, these activated cells release potent growth-regulatory molecules that can act in an autocrine or paracrine manner. Under the influence of cytokines and growth factors, VSMCs adapt to a synthetic phenotype and commence proliferation and migration across the internal elastic lamina into the intimal layer. Stimulated VSMCs allow for the deposition of extracellular matrix, thus converting the initial lesion to a fibrous plaque.

Pathologic examination of human atheromas has identified several growth factors and cytokines that are upregulated in arteriosclerotic patients compared with normal controls [12]. In order for these findings to be clinically meaningful, investigators have attempted to relate markers of systemic inflammation with cardiovascular events. Several markers have received much attention, including fibrinogen, serum amyloid A, and C-reactive protein. The Cholesterol and Recurrent Events study found that higher levels of serum amyloid A and C-reactive protein predicted recurrent coronary events in patients who had myocardial infarction [13]. A recent study prospectively monitored levels of several inflammatory markers in more than 28,000 postmenopausal women [14]. C-reactive protein was associated with the

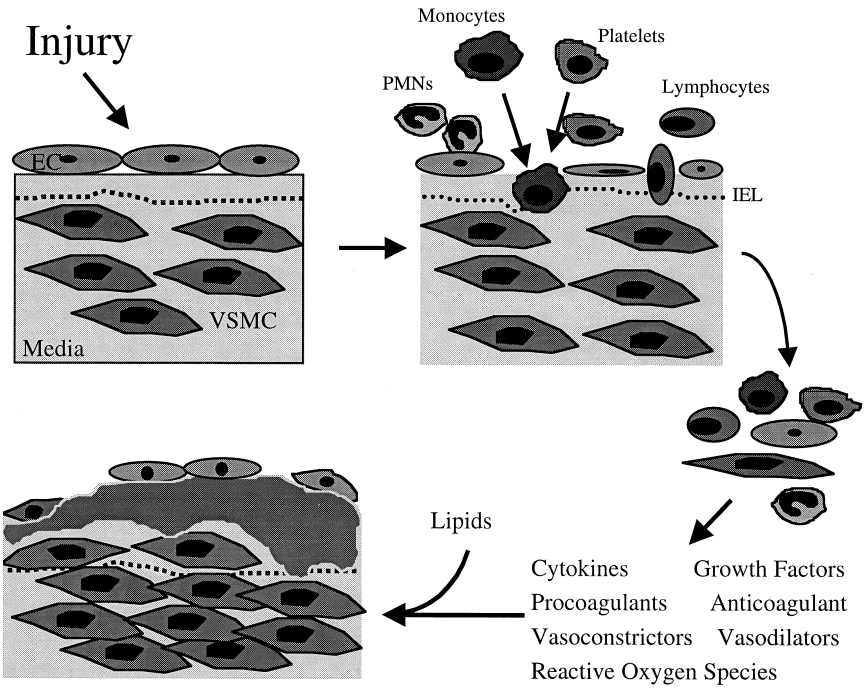

*Fig 1. The response to vascular injury. Therapeutic approaches target one or more points within this paradigm. (*EC = *endothelial cells;* IEL = *internal elastic lamina;* PMNs = *polymorphonuclear neutrophils;* VSMC = *vascular smooth muscle cell.)*

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highest relative risk (fourfold) of having a cardiovascular event. Relative risks greater than twofold were also found with serum amyloid A, soluble intracellular adhesion molecule-1, interleukin-6, homocysteine, apolipoprotein B-100, and total and LDL cholesterol levels. Interestingly, levels of C-reactive protein and serum amyloid A were significant predictors of risk even in patients with LDL levels below 130 mg/dL. Cumulatively, these studies suggest that markers of inflammation, especially C-reactive protein, should be surveyed in high-risk patients, just as one would check a fasting lipid panel.

Modification of Vascular Injury

Therapeutic strategies for cardiovascular disease can try to mitigate the influence of injury or intervene with the vessel's response to that injury. The former approach is founded in the divergent theory; ie, a given injury initiates a cascade of inflammatory events. The latter approach is the basis of the convergent theory; ie, multiple injuries initiate reactions that ultimately lead to a few dominant events. Quite likely both theories are operative. Attempts to limit or eliminate injury will affect atherogenesis, as shown by primary and secondary prevention trials regarding the effects of smoking cessation, control of hypertension, and lipid modification on cardiovascular morbidity and mortality rates. This review will focus on three types of injury that recently have received much attention.

Oxidants

The oxidative-modification hypothesis postulates that atherogenesis is initiated by oxidation of the lipids in LDL [15]. Native LDL trapped in the subendothelial space is oxidized by resident vascular cells. Oxidized LDL induces monocyte chemotaxis, stimulates macrophage uptake of cholesterol with subsequent foam cell necrosis, and promotes elaboration of toxic free radicals. Along with oxidized LDL itself, released reactive oxygen species (including superoxide and hydrogen peroxide) directly injure vascular cells, impair endothelial vasomotor function, promote platelet aggregation or leukocyte adhesion, and stimulate VSMC proliferation.

The oxidative-modification hypothesis is supported by several epidemiologic studies that show an inverse association between the frequency of coronary artery disease and dietary intake of antioxidant vitamins [16, 17]. Whereas observational and experimental studies provide compelling evidence for a beneficial role for antioxidants, randomized prospective trials have had conflicting results. Patients with known coronary disease who received vitamin E had a 77% reduction in nonfatal myocardial infarctions at 17 months [18]. Conversely, in more than 9,000 high-risk patients, vitamin E therapy had no effect on cardiovascular outcomes [19]. Unfortunately, those studies tested different doses of antioxidants. Furthermore, treated patients suffered from advanced levels of arteriosclerotic disease. Oxidation of LDL is an important event early in arteriosclerosis–including the development of the fatty streak. Antioxidant therapy likely needs to be initiated in younger patients to have maximal antiatherogenic effects. Although intuitively attractive, the cause-and-effect relationship of antioxidant therapy and cardiovascular disease remains speculative.

Homocysteine

Homocysteine is an amino acid intermediate formed by the metabolism of methionine, an essential component of animal and plant proteins. Hyperhomocysteinemia can be secondary to genetic enzyme defect (cystathionine β-synthase), vitamin deficiency (folate), chronic medical disorder (renal failure), or medication (phenytoin). Homocysteine is oxidized rapidly in plasma and tissue macrophages. The release of damaging reactive oxygen species initiates the inflammatory cascade. Hyperhomocysteinemia is associated experimentally and clinically with increased platelet aggregation, abnormalities of fibrinolysis, global endothelial cell dysfunction, oxidation of LDL, and proliferation of VSMCs, all of which contribute to atherothrombosis.

The relationship between excess homocysteine and cardiovascular disease was described initially in an autopsy study of children with homocysteinuria and diffuse arterial thrombosis [20]. Subsequently, nearly 30 observational studies have corroborated that association [21]. Compared with normal controls, 35% to 45% of patients with known cardiovascular disease have elevated levels of homocysteine. The relative risk of coronary arterial disease in those patients was estimated to be 24 times that of controls [22]. Thus, hyperhomocysteinemia appears to be an independent risk factor for cardiovascular events. Nearly 10% of the risk of coronary arterial disease in the general population is attributable to homocysteine. Comparatively, an increase of 5 μmol/L in plasma homocysteine concentration (normal 5 to 15 μmol/L) raises the risk of coronary disease as much as an increase of 20 to 85 mg/dL in total cholesterol concentration [23, 24].

Should we all take folate with our vitamin E? Folic acid, vitamins B_{12} and B_6, and pyridoxine are important cofactors for the enzymatic processing of homocysteine. Indeed, the reduction in mortality rate from cardiovascular causes since 1960 has been correlated with the increase in vitamin B_6 supplementation in the food supply. Furthermore, the United States Food and Drug Administration mandates folic acid fortification in flours and cereal products as a method of preventing arteriosclerotic-related death. Although these supplements might effectively decrease serum homocysteine levels, the expected decrease in cardiovascular events has not yet been documented in prospective, randomized clinical trials. Although treatment appears intuitively sound in patients with moderate to severe hyperhomocysteinemia and known cardiovascular disease, universal screening and treatment for the general population is likely premature [25].

Infectious Disease

Infectious diseases have long been associated with atherogenesis. Bacteria include *Chlamydia pneumoniae, Helicobacter pylori, Streptococci, and Bacillus typhosus*; viruses include influenza, herpes virus, adenovirus, and

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cytomegalovirus. Studies to implicate those organisms as directly causative pathogens mostly have been inconclusive [26]. Currently, much enthusiasm exists concerning the relationship between *C pneumoniae* and cardiovascular disease.

The initial epidemiologic description linking chlamydial species to arteriosclerosis was by venerologists in South America in the 1940s. *Chlamydia pneumoniae,* previously referred to as the TWAR strain, is a ubiquitous respiratory organism. More than 50% of the population has antichlamydial antibodies by age 50 years. The prevalence of this organism makes it difficult to determine its true role in arteriosclerosis. In the Arteriosclerosis Risk in Communities Study, which prospectively studied participants who were free of coronary disease at baseline, *C pneumoniae* immunoglobulin G titers were found in only 65% of patients who had subsequent cardiovascular events, compared with 55% in the control cohort [27].

Chlamydia pneumoniae has been detected histologically in patients with end-stage cardiomyopathies, nonrheumatic stenotic heart valves, aortic aneurysms, and carotid endarterectomy lesions. Although these observational studies suggest an association between *C pneumoniae* and arteriosclerosis, it is not known whether this organism is an active participant or an innocent bystander. *Chlamydia pneumoniae* can infect vascular cells, survive, and actively replicate [28]. *Chlamydia pneumoniae* has been associated with decreased levels of HDL cholesterol and increased levels of total cholesterol, thus creating an atherogenic milieu. Furthermore, *C pneumoniae* infection activates inflammatory intracellular transcription factors, promotes cytokine release, and stimulates VSMC proliferation [29, 30]. As such, a scenario can be envisioned where *C pneumoniae* infects macrophages, endothelial cells, and VSMC and promulgates the vascular response to injury.

Should we all take a macrolide with our folate and vitamin E? Is *C pneumoniae* the *H pylori* equivalent for cardiovascular disease? The jury remains undecided. The dynamic nature of arteriosclerosis suggests that eradication of *C pneumoniae* alone would not have the profound effect on disease as seen with *H pylori* and peptic ulcer disease. Currently, several small secondary antibiotic prevention trials have been reported. Patients with unstable angina and male survivors of myocardial infarction treated with macrolides had lower titers of antichlamydial antibody and fewer adverse cardiovascular events [31, 32]. Conversely, in patients with known coronary disease, anti-*C pneumoniae* therapy with azithromycin had no apparent effect on cardiovascular events at 6 months [33]. Conclusions from these studies must be interpreted with several caveats. Each trial enrolled small numbers, examined early outcomes (before 6 months), and gave different doses of antibiotics. Currently, two large trials, which will likely delineate the role of *C pneumoniae*-directed therapy and coronary disease, are underway, the WIZARD trial (weekly intervention with zithromax against arteriosclerotic-related disorders) and the ACE trial (azithromycin coronary events study). Each study plans to enroll more than 3500 patients with coronary artery disease, treat with antibiotics from 3 to 12 months, and observe for 2.5 to 4 years.

Cellular Response to Vascular Injury

Although attempts to modify traditional risk factors have influenced cardiovascular outcomes, how is it possible to control for all the different sources of vessel injury? Vascular remodeling begins at a young age, and, as such, disease processes start long before symptoms become apparent. Convergent theory would suggest that mechanical, metabolic, and toxic insults lead to a common pathway that triggers or potentiates atherogenesis. Thus, therapeutic efforts should interrupt the vessel's response to injury. Both intraluminal and vessel wall components are essential for vascular protection and repair. Understanding the pathophysiologic response of these individual cells is essential for directing therapy.

Endothelial Cells

A healthy blood vessel wall is lined by a monolayer of metabolically active endothelial cells [34]. The surface area of the endothelium is approximately 5,000 m^2 but comprises only 1% of total body weight. While acting as a physical barrier that protects the underlying vessel and allows formed blood elements to flow freely preventing thrombosis, this seemingly bucolic layer is a control center of vascular physiology. The endothelium is an important docking point for monocytes, neutrophils, and lymphocytes by virtue of its ability to express cell-specific adhesion molecules. The endothelium is also a source of mediators that regulate vascular tone. Factors favoring vessel relaxation include nitric oxide and prostacyclin. Factors favoring vessel constriction include thromboxane, leukotrienes, free radicals, endothelins, and cytokines, including tumor necrosis factor and interleukin-1. Finally, endothelial cells produce stimulatory and inhibitory mitogens that act in both an autocrine and paracrine manner (Fig 2).

Circulating and Infiltrating Cells

Along with the resident endothelial and VSMCs, circulating monocytes, neutrophils, platelets, and lymphocytes regulate inflammation. These cells are directed to sites of injury, adhere to or adjacent to endothelial cells, invade the internal elastic lamina (transmigration), release a variety of substances that act on the local environment, and promote chemotaxis. Released cytokines and growth factors produce the effects of inflammation by mediating communication between and among circulating and resident vascular cells. The relationships between the circulating cells and the released cytokines and growth factors define the vessel's ultimate response to injury (Fig 2).

The initial event at sites of vessel injury is the adhesion of circulating cells to endothelial cells, VSMCs, and matrix proteins. These interactions are characterized by a diverse system of surface molecules and receptors broadly categorized as integrins, immunoglobulins, and selectins [35]. When blood monocytes attach and infil-

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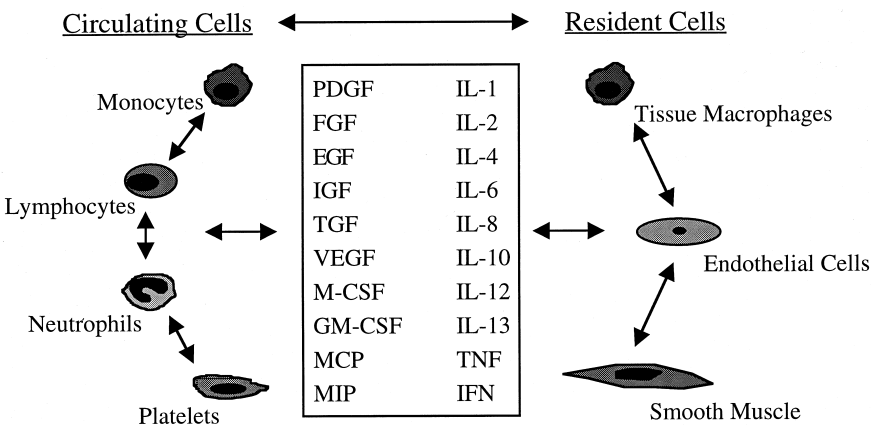

*Fig 2. Growth factor and cytokine relationships that are important in atherogenesis. These families of proteins act in autocrine, juxtacrine, and paracrine fashion to communicate among and between circulating and resident vascular cells. (*PDGF = *platelet-derived growth factors;* FGF = *fibroblast growth factors;* EGF = *epidermal growth factors;* IGF = *insulin-like growth factors;* TGF = *transforming growth factors;* VEGF = *vascular endothelial growth factor;* M-CSF = macrophage colony-stimulating factors; GM-CSF = *granulocyte-macrophage colony-stimulating factors;* MCP = *monocyte chemotaxic proteins;* MIP = *macrophage inflammatory proteins;* IL = *interleukin;* TNF = *tumor necrosis factors;* IFN = *interferons.)*

trate into the vessel, they transform into tissue macrophages. These cells are important in the early formation of the fatty streak and the more advanced atheroma. Although macrophages are a potent source of inflammatory mediators, they are probably best know for their abilities to ingest lipids (phenotypically described as a foam cell) and to scavenge oxidized LDL with production of free radicals.

Both T and B lymphocytes interact with macrophages and have been identified within the atherosclerotic plaque. Immune-mediated disease often reflect an imbalance between proinflammatory and anti-inflammatory responses. T-helper-1 lymphocytes (Th1) promote T-lymphocyte and monocyte activation and produce interferon-γ, granulocyte-macrophage colony stimulating factor, and interleukins 2 and 3. T–helper-2 lymphocytes (Th2) produce interleukins 4 and 10 and can inhibit Th1 responses. The T lymphocyte classically is activated by the presentation of antigen by cellular glycoprotein receptors encoded by the major histocompatability complex. In atherosclerotic plaques, the endothelial cells, smooth muscle cells, and macrophages all express the HLA-DR receptor and activate T lymphocytes [36]. These observations suggest that a local immune response contributes to vascular inflammation and repair.

The identification of a factor released by platelets that stimulated VSMC proliferation was one of the initial observations in support of the inflammatory hypothesis [11]. This substance, platelet-derived growth factor, is now recognized as deriving from multiple cell types, including platelets. Although platelets contribute to the overall inflammatory milieu, they are central to later stages of arteriosclerosis (types V and VI). Thrombosis is central to the pathogenesis of acute arterial insufficiency and acute coronary and cerebrovascular syndromes, including unstable angina, non-Q-wave myocardial infarction, acute (ST-elevation) myocardial infarction, and vessel occlusion after vascular intervention (eg, angioplasty). The three phases of platelet activation include adhesion, aggregation, and secretion. With exposure of the subendothelial space after vascular injury, platelets adhere to basement membrane proteins, especially collagen. This adhesion is dependent upon binding of endothelial or circulating von Willebrand factor to the platelet membrane glycoprotein Ib receptor. The predominant mechanism of aggregation involves binding of fibrinogen to the platelet glycoprotein Ib or IIIa receptor. Platelet secretion usually follows aggregation. Released products include the contents of dense bodies (serotonin, calcium, and adenosine triphosphate) and alpha granules (von Willebrand factor, fibrinogen, growth factors, platelet factor-4, and coagulation factors).

Vascular Smooth Muscle Cells

Classic models of balloon injury showed a central role of VSMCs in neointimal hyperplasia [37]. The three-wave hypothesis suggested that with injury, contractile VSMCs in the media would assume a synthetic phenotype and proliferate. Subsequently, VSMCs would migrate luminally across the internal elastic lamina. Finally, these intimal VSMCs recommence proliferation. Although intimal hyperplasia is histopathologically distinct from atheromatous lesions, this model addresses the importance of VSMC in atherogenesis. Vascular smooth muscle cells are not idle components of the vessel wall. In addition to their contractile properties governing vasomotor tone, VSMCs, like endothelial cells, are an active organ. Importantly, VSMCs are the principal manufacturers of proteoglycans and collagen. Most of the atheromatous lesion is composed of extracellular matrix deposition.

Mitogenic stimulation of VSMCs, with subsequent VSMC proliferation is central to intimal hyperplasia. Interestingly, VSMCs in advanced lesions often are quiescent [38]. This observation underscores the dynamic nature of VSMCs. For example, tumor necrosis factor-α stimulation might promote both VSMC proliferation and apoptosis [39, 40]. How is it that a given cytokine can produce opposite effects? The VSMC response likely depends on interactions with other inflammatory mediators and the stage of vessel remodeling. In type IV and V lesions, the atheromatous core is protected by a thin fibrous cap with edges composed of VSMC. The patho-

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genesis of acute coronary syndromes likely involves apoptosis of these VSMC, thus allowing for rupture of the cap into the lesion with subsequent thrombosis [41]. As such, these disparate biologic responses must be considered when contemplating therapeutic manipulation of VSMC physiology.

Adventitia

As one of only three principal layers of the blood vessel wall, the adventitia has been largely ignored. The adventitia is not simply a structural layer. Adventitial fibroblasts can migrate luminally, secrete mitogens and free radicals, and proliferate, activities that abet atherogenesis [42]. The adventita is the site of vessel sympathetic and parasympathetic innervation. Neurotransmitters released from these efferent fibers assist with vasomotor tone and, importantly, can provide compensatory vasodilation during periods of endothelial dysfunction [43]. Finally, the adventitia is a significant site of resident mast cells. Adventitial mast cells appear to be involved in areas of plaque rupture in acute coronary syndromes [44].

The adventitia is a virtual sponge for protein and gene transfer. Fibroblasts avidly take up viral vectors and can be programmed to enhance nitric oxide production [45]. Periadventital delivery of antisense oligonucleotides to proliferative factors has decreased intimal hyperplasia in vivo [46]. As cardiothoracic surgeons, we see epicardial coronary arteries and diseased peripheral vessels every day. Manipulation of the adventitia is an exciting and feasible area of cardiovascular research.

Intercellular and Intracellular Response to Vascular Injury

Cytokines, Growth Factors, and Inflammatory Mediators

Mediators of inflammation are represented by a diverse group of peptides that function in autocrine, juxtacrine, and paracrine fashions (Fig 2). These substances can arbitrarily be subclassified as growth (platelet-derived growth factor, fibroblast growth factor, epidermal growth factors, insulin-like growth factors, vascular endothelial growth factor), chemokine (monocyte chemotaxic proteins, macrophage inflammatory proteins, interleukin 8), proinflammatory (interleukins 1, 2, 6, 12, 13, interferon, and tumor necrosis factor), and anti-inflammatory factors (transforming growth factor and interleukins 4, 10, and 12). This classification is not meant to be restrictive: most cytokines and growth factors are multifunctional. For example, fibroblast growth factor is a well-known VSMC mitogen [47], but it also promotes adhesion molecule formation and angiogenesis [48].

Cytokines and growth factors regulate leukocyte-endothelial adhesion, chemotaxis, cellular proliferation, apoptosis, and vasomotor tone. These mediators often work synergistically to exert their influence on circulating and resident vascular cells. Although we conveniently group these mediators as cytokines or growth factors, several other substances are inherently involved in the inflammatory response. Endothelins are well recognized as potent vasoconstrictors, but endothelins are also mitogenic for VSMC and promote endothelial cell adhesion molecule expression [49]. Conversely, although prostaglandins promote vasodilation, they also inhibit platelet aggregation and VSMC proliferation. The renin-angiotensin system is often dysfunctional in cardiovascular patients. Similar to endothelins, angiotensin-II is a potent vasoconstrictor, but it also increases adhesion molecule expression and promotes VSMC growth. The antiatherogenic influence of angiotensin blockade, either by converting-enzyme inhibitors or receptor antagonists, appears promising [50, 51]. Cumulatively, the positive and negative interplay between these diverse inflammatory mediators determines the vascular cell's response to noxious stimuli.

Experimentally, anti-growth factor therapies can inhibit intimal hyperplasia [52]. However, because of the functional redundancy among inflammatory mediators, strategies aimed at inhibiting a single substance are unlikely to succeed. As such, researchers have investigated the effects of manipulating intracellular signal transduction on vascular physiology. These strategies exemplify the notion that a variety of insults will initiate a cascade of events that converge to one or more dominant events. As shown in Figure 3, inflammatory mediators engage cell-surface receptors, which sequentially activate a web of intracellular signals. Interactions between intracellular intermediates ultimately might influence gene transcription, protein translation, and cell-cycle regulation.

Nuclear Factor Kappa B and Atherogenesis

Although many intermediates are involved with the stress response, the transcription factor nuclear factor kappa B (NFκB) is a central regulator of inflammatory signals and is upregulated in arteriosclerotic lesions [53, 54]. NFκB will serve as an example of how selective manipulation of intracellular signal transduction can effectively influence the response to injury. NFκB is activated by balloon injury, shear stress, and exposure to oxidized LDL, platelet-derived growth factor, fibroblast growth factor, and tumor necrosis factor [55, 56]. When bound by its inhibitory protein, IκBα, NFκB exists in the cytoplasm as an inactive dimer (p50/p65). Upon stimulation, phosphorylation of IκBα disrupts the NFκB:IκBα complex. Subsequently, NFκB is free to translocate to the nucleus, engage DNA, and initiate gene transcription [57]. Once activated, NFκB promotes many events that are important in atherogenesis (Fig 4).

The effects of many antiatherogenic agents can be explained mechanistically, in part, by inhibition of NFκB activity. Hydroxymethyl glutaryl coenzyme A (HMG-CoA) reductase inhibitors are one of few drugs that have induced regression of arteriosclerosis. Although the -statin drugs effectively decrease total and LDL cholesterol, they are also potent inhibitors of NFκB activity in macrophages and VSMCs [58]. Epidemiologically, moderate alcohol consumption is inversely associated with

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coronary disease [59]. Although substances in red wine have antioxidant properties, flavonoid compounds downregulate NFκB [60]. Finally, aspirin is the most commonly prescribed drug for cardiovascular patients. Although it is best known as an antiplatelet agent, salicylates are excellent NFκB antagonists [61].

Although those diverse therapies likely have multiple points of action, alternative approaches have attacked NFκB activity directly. Overexpression of the inhibitory peptide IκBα, either with transdominant mutants or direct delivery with liposomes, can inhibit VSMC proliferation and other markers of vascular inflammation [39]. Gene therapy strategies have utilized antisense oligonucleotides to the p65 subunit of NFκB to prevent intimal hyperplasia after injury to rat carotid arteries [62]. Cumulatively, these studies show that manipulation of intracellular mediators of inflammation is not only feasible but potentially effective.

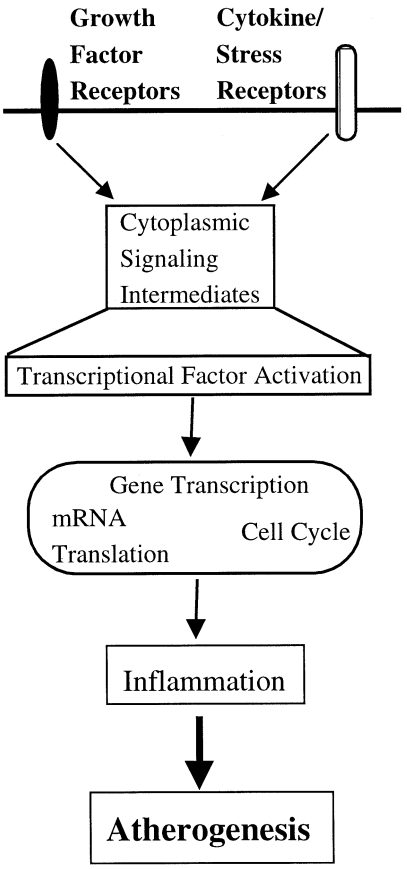

Fig 3. Global intracellular signal patterns involved with atherogenesis. Peptide growth factors, cytokines, and stress activators are mediated by a group of receptors bound by intracellular protein kinases. Receptor-mediated events rely on multiple cytoplasmic signaling intermediates that activate transcription or translational factors. These factors transduce signals to the nucleus, influencing gene transcription, translation, and cell-cycle mechanics. Cumulatively, these signals promulagate the inflammatory and atherogenic response.

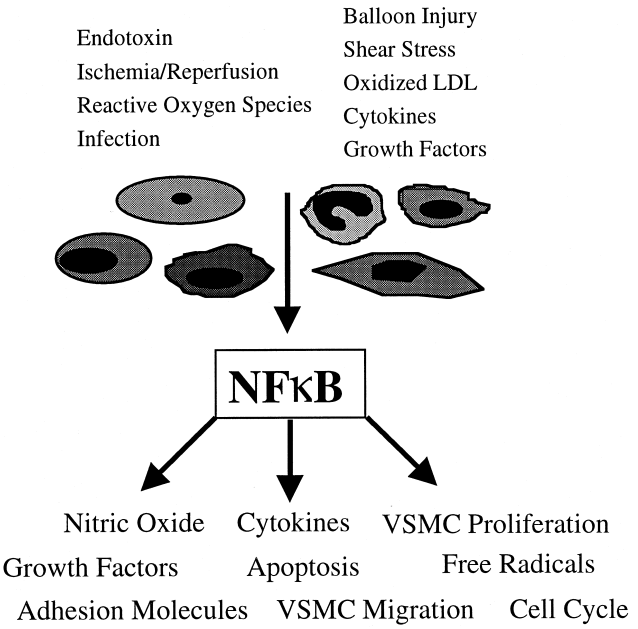

Fig 4. Nuclear factor kappa B (NFκB) and atherogenesis. This intracellular transcription factor is activated by a variety of stimuli, is present in all vascular cells, and is a central mediator regulating multiple atherogenic events. (LDL = low-density lipoprotein; VSMC = vascular smooth muscle cell.)

Atherosclerosis, Inflammation, and Therapy

Figure 1 implies that understanding the pathophysiology of lesion formation will direct our ability to treat this disease. The inflammatory process can be halted or attenuated by intervening at each point in the response to injury. Scenarios of how inflammatory cell-based therapy can be approached for arteriosclerosis are listed by pathophysiologic event. Although many of the following examples have been attempted experimentally, others remain theoretical.

ENDOTHELIAL CELL INJURY. Promote reendothelialization with angiogenic factors (eg, vascular endothelial growth factor).

ENDOTHELIAL DYSFUNCTION. Restore vasomotor tone with nitric oxide donors (eg, L-arginine) and limit toxic damage with antioxidants (eg, vitamin E).

ATTACHMENT OF CIRCULATING CELLS. Block adhesion molecules on leukocytes (anti-CD11/18) and endothelial cells (anti-intracellular adhesion molecule (ICAM).

INFILTRATION OF ADHERENT CELLS. Block leukocyte transmigration (anti-$\alpha_{III}\beta_v$ integrin).

INFLAMMATORY MEDIATORS. Counteract inflammatory mediators with specific (tumor necrosis factor-binding protein) or global (interleukin 10) cytokine antagonist.

CELLULAR ACTIVATION. Manipulate intracellular signal transduction (NFκB).

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VSMC PROLIFERATION. Regulate balance between cell growth (cell cycle proteins) and apoptosis (p53).

COLLAGEN DEPOSITION. Decrease accumulation and organization of extracellular matrix (metalloproteinase inhibitors).

PLAQUE RUPTURE. Regulate cellular apoptosis and fibrous cap stability (integrin and metalloproteinase antagonists).

THROMBOSIS. Inhibit adherence, aggregation, and secretion of platelets (glycoprotein IIb/IIIa receptor antagonists).

Teleologically, one can modify the vascular response by attenuating the unwanted effects while accentuating the desired effects. The functional redundancy within the inflammatory cascade limits attempts to isolate a given cytokine or growth factor. As such, an ideal agent would attack several levels of the response to injury. Experimentally, the anti-inflammatory cytokine, interleukin-10 meets several of these criteria. Interleukin-10 downregulates adhesion molecule expression, inhibits tumor necrosis factor and interleukin-1 release by vascular cells, and inhibits VSMC proliferation [63, 64]. Currently, it is not known whether interleukin-10 can beneficially influence vascular remodeling in vivo. Several studies found the protective effects of estrogens on cardiovascular disease in postmenopausal women. Estrogens attack each level of the inflammatory response;eg, mitigating the "bad" mitogenic influence of growth factors on VSMC and exaggerating the "good" nitric oxide production by the endothelial cell [35].

In conclusion, antiatherosclerosis strategies aim to eliminate injury or the vessel's pathologic response to that injury. Primary prevention remains the most feasible option. Beneficially modifying traditional cardiovascular risk factors synergistically attenuates cumulative injury. Interestingly, many of the drugs used to treat hyperlipidemia or hypertension are actually anti-inflammatory drugs themselves (eg, statins and calcium-channel blockers). Smoking cessation alone will not eliminate cardiovascular disease. Within the current paradigm, inflammation and injury are dynamic events. Therapy must also be directed at the smoldering inflammation ongoing in the vessel wall.

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