Review

Inflammation at the Molecular Interface of Atherogenesis

An Anthropological Journey

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Despite the multifactorial nature of atherosclerosis, substantial evidence has established inflammation as an often surreptitious, yet critical and unifying driving force which promotes disease progression. To this end, research has defined molecular networks initiated by cytokines, growth factors and other pro-inflammatory molecules which promote hallmarks of atherosclerosis such as endothelial dysfunction, macrophage infiltration, LDL oxidation, cell proliferation and thrombosis. Although commonly associated with risk factors such as dyslipidemia, diabetes and hypertension, the global etiology of atherosclerosis may be alternatively attributed to underlying anthropological pressures. The agricultural, industrial and technological revolutions produced alterations in dietary, social and economic factors which have collectively exaggerated the exposure of the human genome to environmental stimuli. Furthermore, advances in sanitation, nutrition, and medicine have increased the lifespan of humans, effectively prolonging blood vessel exposure to these factors. As a result, the vasculature has become conditioned to respond to injury with what is arguably an overzealous immunological response; thus setting the stage for the prevalence of cardiovascular disease, including atherosclerotic plaque development in Western populations. Evidence suggests that each of these alterations can be linked to specific mediators in the inflammatory process. Integration of these factors with an inflammation-based hypothesis of atherosclerosis has yet to be extrapolated to observations in the realms of basic and clinical sciences and is the focus of this review. (*Am J Pathol* 2008, 173:1253–1264; DOI: 10.2353/ajpath.2008.080442)

Traditional views of atherosclerosis as simply a lipid-based disorder have been modified by an appreciation for the multifactorial etiology of this disease. Perturbations in vessel wall integrity produce a volatile state in which the collective identity of cellular players and their associated molecular mediators is altered. The societal burden of these events is evident in light of the fact that an estimated 80 million American adults suffer from one or more types of cardiovascular disease (CVD).¹ Classical risk factors such as hypertension, diabetes, and obesity are useful as predictors of atherosclerotic disease; however, they alone cannot unequivocally define its pathogenesis. Similarly, whereas the usefulness of lipid profiling is indisputable, cardiovascular events occur in patients with plasma cholesterol concentrations below recommended guidelines. To this end, the establishment of a unifying fundamental mechanism accompanied by a characterization of relevant underlying stimuli may provide the basis for a novel assessment of atherogenesis.

A tremendous amount of evidence supports a role of inflammation in propagating the transition from early atherogenesis to thrombotic events associated with myocardial infarction or stroke. Accordingly, the use of leukocyte count, C-reactive protein (CRP), interleukin (IL)-18, IL-6, and soluble CD40 ligand as inflammatory markers of disease has become a useful tool for clinicians.² Although a straightforward characterization of atherogenesis as an inflammatory process is essential to a greater understanding of relevant pathological mechanisms, the evolution of this paradigm is equally useful and has yet to be examined in this context. Although the term environmental is often applied to describe influences of

Supported by the National Institutes of Health (grants to PO1 HL46403, PO1 HL072942, and T32 HL07423 to D.P.H.), the Abercrombie Foundation, and the Julia and Seymour Gross Foundation.

Accepted for publication August 5, 2008.

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our surroundings on biological characteristics, an anthropological viewpoint offers a unique and perhaps less restrictive panorama into the origins of human pathophysiology. The present review first assembles current data supporting the role of inflammation throughout the development and materialization of atherosclerotic disease. Subsequently, an anthropological lens is used to clarify the conceptual foundations of atherogenesis while highlighting inflammation at the molecular interface of these phenomena. Extrapolation of this point of view on both a scientific and clinical level may shed new light on this disease and potentially stimulate novel approaches for therapeutic interventions.

Interweaving Inflammation and Atherosclerosis

Establishment of the Inflammatory Hypothesis

Inflammation-based arterial changes as a mechanism of primary importance in atherogenesis was originally proposed in the mid-19th century by Rudolf Virchow.³ Since this seminal proposal, many hypotheses have emerged to incorporate the role of inflammation in this disease, such as Russell Ross's commonly cited "response to injury" model of atherogenesis.⁴ Under this model, injury to the endothelium due to mechanical trauma, sheer stress, infection, and/or an increase in reactive oxygen species (ROS) is believed to be the initial trigger for a local inflammatory response.^{4,5} The inflammatory mediators that direct, perpetuate, and magnify disease progression can be categorized by both cell type(s) of origin and temporal relevance to disease stage (Figure 1).

Identification of Inflammatory Targets: Cast of Characters and Mediators in the Vessel Wall

The orchestration of processes associated with plaque formation is primarily performed by endothelial cells (ECs), smooth muscle cells (SMCs), platelets, lymphocytes, monocytes, and macrophages. In a mutually interactive manner, these cells generate a broad range of molecules including cytokines, growth factors, eicosanoids, proteases, and ROS, which provoke an acute and eventually chronic inflammatory condition in the vessel wall. Importantly, many of these molecular mediators are produced by more than one cell type, allowing for substantial cross-pollination of the inflammatory milieu.

Endothelial Cells

In healthy vasculature, the endothelial lining serves as a physical barrier and possesses intrinsic homeostatic functions that deter leukocyte adherence. Vascular fluidity is primarily maintained via the production of antithrombotic molecules including nitric oxide (NO), prostacyclin (PGI₂), and the ecto-ADPase, CD39.⁶ Endothelial injury and activation beget endothelial dysfunction, as low-density lipoprotein (LDL) accumulation and oxidation (in con-

junction with macrophage accretion discussed below) create the hallmark fatty streak in the vessel wall.⁷ CD36, SR-A, and other scavenger receptors allow for the engulfment of modified lipoproteins, namely acetylated (acLDL) and oxidized (oxLDL) LDL via receptor-mediated endocytosis.^{8,9} The formation of oxLDL is believed to play a pivotal role in amplifying the inflammatory response as proinflammatory cytokines promote the attachment, rolling, and infiltration of leukocytes into the intimal layers.^{10,11} Although the initial events following endothelial injury resemble an innate immune response to modified LDL, it is apparent that characteristics of the adaptive immune response also resonate in a chronic setting. Facilitators expressed on activated vascular cells including intracellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM), the P and E selectins, integrins, and chemokines such as monocyte chemoattractant protein (MCP)-1 dictate the pattern of adherence and infiltration of circulating inflammatory cells. The importance of these gate keepers is evident in that pharmacological inhibition or genetic knockdown of these molecules and/or their receptors reduces lesion size, macrophage infiltration, and disease progression in experimental models of atherosclerosis.^{12,13}

Seroepidemiological and in many cases direct physical evidence of infection within lesions led to the proposal of an atherogenic role for *Chlamydia pneumoniae*, cytomegalovirus, herpes viruses, *Helicobacter pylori*, hepatitis, *Trypanosoma cruzi*, and periodontal infections.^{14–18} Although pathogen-driven atherogenesis appears to be mechanistically diverse, the endothelium may be a significant target of infection.⁵ In addition to damage of the endothelial lining, infectious agents promote a pro-adhesive and procoagulant surface that is critical toward the initiation of atherosclerotic disease.¹⁹ Despite promising *in vitro* and animal-model data, however, clinical inconsistencies remain such as the lack of apparent benefits with antibiotic treatment in the secondary prevention of coronary events.²⁰ Nonetheless, the endothelial lining has emerged as the primary target of a variety of pathological stimuli in early-stage, inflammatory-based vascular disease.

It should be noted that a healthy endothelium has the ability to repair its intimal layer following injury. Experimentally, the loss of contact inhibition between ECs results in migration and proliferation of these cells to restore continuity to the endothelial lining.²¹ Circulating, bone marrow-derived, endothelial progenitor cells (EPCs) were demonstrated to mobilize in response to ischemia or cytokine-stimulation and to colonize foci of revascularization.²² Adhesion molecules such as ICAM, as well as soluble mediators, regulate interactions between EPCs and local ECs to resuscitate vascular integrity.²³ EPCs and other circulating progenitor cells are a relatively novel area of research, and as such, caution is warranted in the interpretation of these data. Although there is clear evidence that these cells can repair sites of EC damage and promote the restoration of blood flow under select conditions, other studies have demonstrated that potential benefits may be highly dependent on the source of progenitor cells (bone marrow versus spleen), specificity

Influence of Anthropological Stimuli on Atherogenesis

Figure 1. ECs, macrophages (Mφ), SMCs, platelets, and B and T cells contribute to plaque development, potentially culminating in thrombosis. Vascular events depicted here are induced by inflammatory stimuli and occur in a cyclic manner to drive atherogenesis. Anthropological stimuli intersect at multiple stages of lesion formation to enhance the inflammatory process during atherosclerosis. ECM, extracellular matrix.

of cell-cell interactions, the appropriate stimulation by molecular mediators (growth factors, cytokines), and physiological state of the vessel (healthy versus diseased tissue).²⁴ Furthermore, EPCs have been implicated in tumor vascularization and growth.²⁵ Thus, elucidation of molecular networks regulating EPCs is critical to selectively harnessing their therapeutic potential.

Macrophages

Once integrity vessel wall is compromised, the ability of the vascular microenvironment to control cellular and subcellular events, in particular those associated with inflammation, becomes strained. Following endothelial injury, phagocytic monocyte-derived macrophages penetrate the intima and aid in the oxidation and subsequent accumulation of lipids, further catalyzing the influx of inflammatory mediators. In addition to abetting monocyte/macrophage maturation, MCP-1 increases scavenger receptor expression, thereby perpetuating this cycle.²⁶ Proliferation of macrophages with subsequent alterations in cellular morphology and the formation of foam cells within the fatty-streak leads to enhanced secretion of IL-1 β and tumor necrosis factor (TNF)- α . TNF- α , a systemic mediator

of inflammation linked to proliferation, apoptosis, and differentiation, binds TNF receptor-1 and -2 to activate components of the mitogen-activated protein kinase pathways and increase pro-inflammatory transcription factors such as nuclear factor- κ B (NF- κ B) (discussed below).²⁷ Activated macrophages and other cell types secrete growth factors such as platelet-derived growth factor, epidermal growth factor, fibroblast growth factor, and transforming growth factor, which promote SMC proliferation and further amplify the inflammatory environment.²⁸

Smooth Muscle Cells

As lesions progress to intermediate and eventually mature fibrous plaques, associated fundamental processes remain primarily driven by inflammation. In response to growth factors, SMCs derived from the vessel wall migrate and proliferate throughout the intima.²⁹ In addition to the production of collagen and other extracellular matrix proteins, SMCs secrete vascular endothelial growth factor, TNF- α , IL-1, and other pro-inflammatory molecules.³⁰ Fibrous tissue and proliferating SMCs overlay the mature lipid core, which becomes rich in necrotic debris. The phenotype of SMC migration and proliferation

is a critical component of plaque stability, and, interestingly, the lack of SMCs may paradoxically increase the occurrence of arterial thrombosis.³¹

Platelets

The role of platelets in atherogenesis is believed to be primarily relevant following their exposure to the subendothelial layers consisting of extracellular matrix proteins such as von Willebrand factor and collagen. Upon adherence of these factors to glycoprotein receptors GPIb/IX/V and GPIIb/IIIa, respectively, platelet activation and binding to integrin receptors promote further platelet recruitment through fibrinogen- and fibronectin-mediated interactions. These processes culminate as platelets form an irreversible, tightly compressed mass that serves as the nidus for an occlusive lesion. Importantly, platelets are a repository of inflammatory substances that influence all aspects of disease progression, including adhesion and aggregation (von Willebrand factor, fibrinogen, selectins, thrombospondin), chemotaxis (platelet activating factor-1 and macrophage inhibitory protein-1 α), proliferation (platelet-derived growth factor, transforming growth factor- β), proteolysis [matrix metalloproteinase (MMP)-2], and coagulation (factor V/XI, protein S).³² The metabolic relationship between hemostasis, thrombosis, and atherosclerosis was originally demonstrated by the production of leukotriene B₄, a potent proinflammatory substance released by activated platelets.³³ Inflammatory mediators additionally induce the expression of tissue factor, promoting thrombus formation upon plaque rupture.

Throughout disease progression, silent plaque rupture and thrombosis may occur repeatedly without symptoms. However, these cycles ultimately serve to enhance fibrotic healing, SMC proliferation and to further amplify the inflammatory response. Paradoxically, repetitive cycles of inflammation and resolution may set the stage for a cataclysmic cardiovascular event. In fact, anti-inflammatory mechanisms are continuously evoked to maintain equilibrium. Anti-inflammatory cytokines IL-4, IL-10, and IL-13 may delay plaque formation by interfering at multiple time points during atherogenesis. For example, IL-10 has been demonstrated to suppress NF- κ B activity and associated pro-inflammatory cytokine production in RAW 264.7 macrophages via a reduction in ROS generation.³⁴ In addition to the role of platelets in thrombosis, animal models of atherosclerosis have demonstrated platelet adherence in the absence of endothelial injury and lesion development, suggestive of a role for these cells in the earlier stages of disease.³⁵

ROS: Feeding and Exacerbating Inflammation

Superoxide ($O_2^{\cdot-}$) generation is central to the formation of reactive intermediates including peroxynitrite (ONOO $^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH $^{\cdot}$). In addition to vascular sources of $O_2^{\cdot-}$ such as mitochondrial oxidases, myeloperoxidase, xanthine oxidase, lipoxygenase, and nitric oxide synthases, NADPH oxi-

dase-derived $O_2^{\cdot-}$ is considered to be a particularly critical source of ROS in macrophages.^{28,36} Furthermore, cytokines serve to enhance ROS production in these systems. For example, macrophage colony-stimulating factor (M-CSF), which regulates proliferation, differentiation, and chemotaxis of macrophages, has been shown to stimulate raft-associated NADPH-oxidase allowing for NADPH-oxidase assembly and ROS production.³⁷ Regardless of the cellular and enzymatic source of ROS, these molecules accelerate atherogenesis via oxidation of accumulated LDL and enhanced foam cell formation. It is interesting to note that oxLDL can lead to further production of ROS.³⁷ Although much less common than their negative counterparts, positive feed-back mechanisms such as that observed between oxLDL and ROS are commonplace in the inflammatory processes discussed herein and significantly contribute to pathological phenotypes associated with an otherwise protective biological response. In a similar example, a self-propagating phenomenon involving H_2O_2 and NADPH oxidase was recently described and proposed to contribute to the development of vascular disease.³⁶

ROS also play a role in the regulation of NF- κ B, a ubiquitous nexus in inflammatory and atherogenic pathways. Although numerous transcription factors have been implicated in atherogenesis, NF- κ B controls more than 160 gene products including those involved in cell proliferation, apoptosis, and inflammation.³⁸ NF- κ B is negatively regulated by the inhibitor of kappa B (I κ B), which masks nuclear localization signals essential for translocation from the cytosol to the nucleus. Multiple reports have implicated ROS-driven induction of NF- κ B by oxLDL and H_2O_2 , possibly via inhibitory actions on I κ B.³⁹ Conversely, agents activating NF- κ B are associated with enhanced production of ROS.⁴⁰

NF- κ B promotes the synthesis of numerous proinflammatory cytokines as well as cyclooxygenase (COX)-2 and MMPs.³⁸ COX isoforms catalyze the oxygenation and transformation of arachidonic acid into eicosanoids that possess both pro-(PGE₂, TXA₂) and anti-(PGI₂) inflammatory characteristics; the balance of which may have critical influences on vascular equilibrium.⁴¹ For example, COX-2-derived PGE₂ stimulates EP-4 receptor-mediated enhancement of inflammation in atherosclerotic plaques as well as induction of MMP-9 expression, a process that contributes to plaque destabilization in late-stage disease.^{42,43}

Although there is much debate as to the clinical usefulness of antioxidants, experimental evidence has shown that a reduction in oxidative stress is beneficial in animal models of disease. Lipoic acid, an organic compound associated with numerous mechanisms of action, was found to suppress atherosclerosis in both apolipoprotein E (ApoE)-deficient and ApoE/LDL-receptor-deficient mice.⁴⁴ Similarly, the antioxidant actions of many commonly used cardiovascular drugs were demonstrated to target redox-sensitive transcription factors and to reduce the expression of VCAM-1, ICAM-1, and selectins in human aortic ECs.⁴⁵ It would appear that the class of antioxidant, its dosing regimen and extent of disease may govern the effectiveness of these agents. Recently,

Table 1. Molecular Mediators of Inflammation Provoked by Anthropological Risk Factors as a Result of the Agricultural and Industrial Revolutions

Anthropological risk factor	Influence of agricultural and industrial revolutions	Associated molecular mediators of inflammation (eg)
Energy intake	↑	TNF- α , CRP, ROS
Total/saturated fat/trans fat	↑↑	TNF- α , COX-2, PGE ₂ , MMP, ROS
ω -6: ω -3 ratio	↑↑	ICAM, VCAM, selectins, PDGF, MMP, TXA ₂ , ROS, ↓ NO
Salt	↑↑	MCP-1, PAI-1, COX-2, ROS
Smoking	↑↑	ICAM, TNF- α , COX-2, PGE ₂ , ROS
Vitamins (A, E, C)	↓	ROS, IL-8, PAI-1
Physical activity	↓	Cytokines, EPC, adiponectin

TNF, tumor necrosis factor; CRP, C-reactive protein; ROS, reactive oxygen species; COX, cyclooxygenase; MMP, matrix metalloproteinase; MCP, monocyte chemoattractant protein; ICAM, intracellular adhesion molecule; VCAM, vascular cell adhesion molecule; PDGF, platelet-derived growth factor; NO, nitric oxide; PAI-1, plasminogen activator inhibitor; IL, interleukin; EPC, endothelial progenitor cell.

the development of source-specific inhibitors of ROS production, such as mitochondria-targeted antioxidants that concentrate on the inner mitochondrial membrane, appear to confer exceptional protection against mitochondrial oxidative damage in atherosclerosis.⁴⁶

Compelling evidence supports an intimate association between inflammation and atherosclerosis, and ROS likely represent a pivotal link between these two processes. Some have proposed multiple modes of inflammation in atherogenesis, perhaps highlighted by fluctuations in inflammatory mediators.⁴⁷ Nevertheless, it is interesting to speculate i) how a presumably protective event such as an immune response is linked to the number one cause of death (CVD) in industrialized nations, ii) why our genetic evolution has been unable to prevent or minimize the impact of inflammation in perpetuating atherosclerosis, and iii) what are the fundamental impetuses and mechanistic links to the development of these phenomena?

Foundations of Inflammation

Risk factors such as dyslipidemia, diabetes, obesity, and hypertension may be viewed as products of broader historical influences. Recent work has described a genetic rift between modern-day humans and our ancestors as a result of widespread changes in diet, physical activity, and other factors.^{48,49} Before and throughout the 2 million years encompassing the Paleolithic Age, environmental pressures (such as the availability of food sources) combined to select those individuals, including their respective genotypes, which were most suitable for survival and reproduction. The agricultural revolution as well as the more recent industrial and technological revolutions provoked robust changes in dietary, social, and economic factors. It is important to stress that although documentation regarding anthropological factors throughout the last few hundred years are available and provided as tangible data, many of the more historical trends have been estimated from a variety of sources including the extrapolation of data on contemporary hunter-gatherer populations. Other influences such as the evolving impact of pathogens on atherogenesis are extremely difficult to extrapolate throughout time periods. Nonetheless, it would appear

that because of the relatively rapid nature of these alterations, our intrinsic vulnerability to the robust responsiveness of our immune systems has become exposed. We propose that as a result, many of these factors can be correlated to specific molecular mediators (Table 1) and to one or more stages of plaque development (Figure 1).

Rapid Evolution of Diet

Revolutions

Although varying by geographical local, pre-agricultural hunter-gatherers depended primarily on diets consisting of uncultivated plant foods (fruits and vegetables) with sporadic consumption of wild game (lean meat) and fish.⁵⁰ Approximately 10,000 years ago, the agricultural revolution led to increased consumption of fat-laden meat and dairy products from domesticated animals and large amounts of cereal grain.⁵¹ These dietary changes have been amplified in the last 200 years following the industrial and technological revolutions as the mass production of food sources and food-processing techniques have conspired to create the modern Western diet.⁵⁰ The so-called classical Western diet of developed countries, notably that of the United States, is characterized by high intake of red meat, fat, cereal grains, and refined sugars.^{50,51} Examination of some of the major components in our diets reveals underlying stimuli for an enhanced immunological response relevant to vascular disease.

Fatty Acids

The type and relative proportions of fatty acid ingestion are believed to play a significant role in maintaining a healthy equilibrium in the vasculature.^{52,53} Fatty acids can be categorized as saturated (SFA) or unsaturated, with the latter falling into the poly- (PUFA) or mono-unsaturated varieties. PUFAs are subdivided primarily into ω -6 and ω -3 fatty acids. Unsaturated fatty acids from nonadipose tissue comprise a considerable portion of total fatty acids in wild game.⁵⁴ Conversely, fattening techniques associated with the domestication of animals, food processing methods (SFA storage in the form of cheese, butter, and so forth), and other advances have generated food sources and thus dietary patterns char-

Figure 2. Hypothetical scheme of average life span at birth and fat intake (as percentage caloric intake) for select time periods. Data adapted from references.^{1,40,46}

acterized by elevated total, saturated, and trans fat as well as an increase in the ω -6: ω -3 ratio.^{49,54,55} Because of the historical trend of increased life expectancy, the impact of changes in anthropologically relevant stimuli has been amplified as vascular segments are simply exposed to these factors throughout an elongated period of time (Figure 2).^{1,51,56} Not surprisingly, diets with elevated total and saturated fat are used to experimentally induce and accelerate atherogenesis in animals. Furthermore, experimental and clinical evidence has demonstrated that a diet high in saturated fat leads to a reduction in LDL receptor-mediated clearance of LDL.^{57,58} In addition to the direct effects of SFAs on lipid profiles, evidence has emerged implicating these molecules in the promotion of inflammation. For example, palmitic acid, a common SFA found in animals and plants, was recently shown to increase NF- κ B DNA-binding activity and TNF- α production by adipocytes.⁵⁹

An increase in PUFA intake in modern diets has resulted in an ω -6 to ω -3 fatty acid ratio that is estimated to have increased fivefold over pre-agricultural levels.⁵¹ Although blood levels of PUFAs correlate directly with the risk of sudden death in patients without previous history of CVD, considerable evidence supports the notion that the ratio, rather than the absolute levels of these fatty acids, is critical as a predictor of atherogenic risk.⁶⁰ Furthermore, it would appear that this ratio has many consequences in terms of the inflammatory response. Lean game meat, wild fruits, flaxseed, nuts, and fish are a rich source of ω -3 fatty acids such as eicosapentaenoic acid and docosahexaenoic acid. These molecules are precursors to eicosanoids that reduce inflammation and serve to mitigate the synthesis of pro-inflammatory eicosanoids from the metabolism of arachidonic acid and other ω -6 fatty acids.⁶¹

Fish oil is a prominent source of ω -3 fatty acids and has been demonstrated to exert a suppressive effect on the production of inflammatory molecules linked to atherogenesis. In fact, the beneficial effects of ω -3 fatty acids are evident by an observed reduction in CVD among Japanese populations consuming large quantities of fish.⁶² Fish oil was also shown to enhance endothelial-

derived relaxing factor (NO) release from ECs, harnessing the potent anti-inflammatory actions of this gas.⁶³ Eicosapentaenoic acid and docosahexaenoic acid additionally function to reduce platelet-derived growth factor, thereby suppressing proliferation and migration of sub-intimal cell types.⁶⁴ Eicosapentaenoic acid administration reduced atherosclerosis in ApoE^{-/-} mice due to attenuated expression of adhesion molecules VCAM-1, ICAM-1, and MCP-1 and attenuation of MMP-2 and MMP-9 induction by macrophage-like cells stimulated with TNF- α .⁶⁵ MMPs are zinc-dependent endopeptidases that in addition to degrading extracellular matrix may also target a number of proteins and regulate cell proliferation, migration (adhesion/dispersion), differentiation, angiogenesis, and apoptosis.⁶⁶ Furthermore, MMP-mediated degradation of extracellular matrix facilitates SMC migration and is critical to macrophage infiltration. In contrast to fish oil, grains are rich sources of ω -6 fatty acids and are low in ω -3 fatty acids. Therefore, the mechanisms attributed to the ability of ω -3 fatty acids to suppress inflammation include the replacement of corresponding ω -6 pools in membrane phospholipids, as well as a reduction in proinflammatory transcription factors, cytokines production, adhesion molecules, chemokines, and ROS.⁶⁷

Interestingly, the chemical identity of PUFAs may also have profound effects on the action of these molecules. For example, PUFAs containing oxygen or sulfur in the β -position exhibit significant inhibition of agonist-induced E-selectin, ICAM-1, and VCAM-1 expression, with a resultant reduction in leukocyte adhesion to cultured human umbilical vein ECs.⁶⁸ Furthermore, a novel PUFA, β -oxa-23:4n-6, inhibited acute and chronic inflammatory responses in mice as well as adhesion molecule induction in the arterial endothelium. These findings were demonstrated to be dependent on metabolism of this fatty acid by 12-lipoxygenase and mechanistically attributed to a suppression of I κ B and ROS production.⁶⁸ Novel PUFAs such as these may represent potential areas of interest for pharmaceutical targeting.

Carbohydrates

Refined grain and sugars are characterized by a particularly high glycemic index compared to traditional sources of carbohydrates such as fruits and vegetables. The prevalence of diabetes and related disorders in Western populations has likely been propelled by the widespread transition to the former as a major component of Western diets. Furthermore, an increase in dietary glycemic index, an indicator of carbohydrate content, is associated with markers of inflammation such as CRP.⁶⁹ The link between glucose and inflammation may also lie in the ability of this monosaccharide to generate ROS via a variety of mechanisms.⁷⁰

When compared to diets relying more heavily on wild plants, those with high grain intake are associated with reduced levels of ascorbate, carotene, thiamine, folate, riboflavin, and vitamins A and E.⁷¹ These molecules serve as essential co-factors (ie, folate) and antioxidants

(ie, vitamins E and C) that, when deficient, may further contribute to injury under conditions of inflammatory-driven oxidative stress in the vascular wall.^{72,73} Vitamin C or (ascorbic acid) has been shown to provide widespread protection against atherogenesis such as the prevention of endothelial dysfunction, inhibition of dedifferentiation, and proliferation of SMCs, and a reduction in oxLDL uptake and degradation by macrophages.⁷⁴ Similarly, vitamin E (tocopherols and tocotrienol derivatives) has been shown to reduce inflammatory markers such as CRP and suppress the release of proinflammatory cytokines plasminogen activator inhibitor (PAI-1) and IL-8.⁷⁵ Despite a wealth of promising evidence in cell and animal studies, a clear link between vitamin intake and the prevention and/or reversal of atherosclerosis is lacking in clinical trials. It has been suggested that optimal protection with vitamins occurs through targeting of the earliest inflammatory changes in atherosclerosis; thus, their ineffectiveness in the reversal of advanced disease may not be surprising.⁷⁴ Based on the vast number of potential targets uncovered by basic science research, however, resolution of conflicting data regarding the role of vitamins as tools for therapeutic intervention in patients with CVD is warranted.

Other Dietary Considerations

Although perhaps less clearly defined, a variety of other dietary alterations may have led to modification of the inflammatory response and associated prevalence of vascular disease. In addition to shifts in dietary constituents throughout the last 10,000 years, total energy and caloric intake have increased.^{50,51} Considerable clinical and experimental evidence suggests caloric intake is inversely proportional to cardiovascular health. As such, caloric restriction improves major atherosclerotic risk factors and reduces markers of inflammation such as CRP and TNF- α .^{76–78} Caloric restriction was also found to reduce ROS-mediated damage of macromolecules resulting in lower levels of oxidized LDL and a reduction in age-related increases in the sensitivity of vascular cells to these molecules.^{79,80} The impact of protein intake on cardiovascular health is unclear as it is often accompanied by high levels of fat. Nonetheless, percent protein intake has decreased in contemporary human populations and dietary protein has been shown to be inversely proportional to blood serum homocysteine, a risk factor for CVD.⁸¹ Finally, high salt intake can be directly correlated to an elevation in markers of inflammation such as COX-2, MCP-1, and PAI-1, as well as increased generation of ROS.⁸²

Socioeconomic Influences

Although the underlying causes of this phenomenon have not been fully determined, lower socioeconomic groups display an increased prevalence of CVD. A recent report attempted to link socioeconomic status to classic markers of inflammation. Using data from the Multi-Ethnic Study of Atherosclerosis, researchers found that lower

household income and educational background were associated with increased blood levels of CRP and IL-6.⁸³ Although limitations exist in the estimation and subsequent analysis of factors such as stress and psychological dynamics across time periods, psychological stress has been proposed to have a particularly strong influence on 21st century humans. Accordingly, neuroendocrine-mediated increases in inflammatory cytokines may play an etiological role in the promotion of inflammatory pathologies.⁸⁴ Further highlighting the interplay between inflammation and oxidative damage, numerous correlations between overall levels of stress and the generation of ROS have been demonstrated.⁸⁵

Problematically, factors such as economic position, education, and stress are difficult to extrapolate to the bench top. On the other hand, examination of parameters such as physical activity and smoking, allow for a more tangible analysis of inflammation as a unifying theme in atherosclerosis. With respect to their historical timelines, we believe that these parameters represent a chronic (reduction in physical activity) and an acute (societal introduction to smoking) example of alterations that have magnified the inflammatory response and driven the epidemic of atherosclerosis.

Physical Activity

Despite the fact that the genetic code of contemporary humans is that of a nomadic hunter-gatherer, our average energy expenditure is estimated to be less than 50% of that of our predecessors.⁸⁶ Although it is not surprising that a sedentary lifestyle produces a greater risk for CVD and an increase in physical activity is recommended in the prevention and treatment of associated conditions, the direct role of an overactive immune system has only recently been appreciated.⁸⁷ ApoE-deficient mice undergoing exercise training were recently found to exhibit a decreased susceptibility to atherosclerotic disease.⁸⁸ Interestingly, the protective effects were attributed to a reduction in ROS.⁸⁸ In another study, physical activity was shown to increase the production and circulating numbers of EPCs in mice.⁸⁹ As described previously, EPCs are being investigated as an important component of endothelial regeneration and compensatory angiogenesis, functioning to counteract and protect the vascular wall from inflammatory cytokines.⁹⁰ In fact, EPC depletion has been associated with numerous factors including hypercholesterolemia, excessive caloric intake, obesity, diabetes, and smoking.⁹⁰ In related phenomena, obesity and the presence of excessive adipose tissue were positively linked to chronic inflammatory conditions. As an active endocrine and paracrine organ, adipose produces inflammatory molecules such as leptin, adiponectin, IL-6, and TNF- α .^{91,92} A reduction in physical activity is thus proatherogenic and can be attributed in part, to an enhancement of inflammatory pathways, a correlation that has recently been proposed in the field of hypertension research.⁹³

Smoking

Since the early days of the American Revolution, tobacco use and cigarette smoking have enormously impacted the development of CVD and atherosclerosis. Smoking is associated with an increased risk of plaque formation and a reduction in plaque stability. Although there is some debate as to the toxins in cigarette smoke that are particularly noxious, previously unrecognized associations of these chemicals with inflammation have now begun to emerge. Acrolein, a toxin found in cigarette smoke, induced COX-2 expression and PGE₂ production in mice. Furthermore, COX-2 and acrolein were observed to co-localize in human atherosclerotic lesions.⁹⁴ Cigarette smoking has also been demonstrated to increase ICAM-1 and TNF- α , promoting endothelial dysfunction and the amplification of inflammatory stimuli.⁹⁵ A novel and intriguing mechanism linking these factors with atherogenesis is protein carbamylation. It was observed that an inflammation-driven reaction whereby myeloperoxidase-catalyzed modifications alter protein function *in vivo*, and is particularly relevant in human atherosclerotic lesions.⁹⁶ Thus, smoking increases markers of inflammation and promotes plaque instability via multiple mechanisms of action; findings that may be correlated to the instability of atherosclerotic plaques in smokers.⁹⁷

Evolution of CVD

Although the majority of historical data regarding CVD is limited to that acquired throughout the past century, paleopathology has provided evidence that patterns of vascular inflammation and atherogenesis existed in more primitive populations. Furthermore, these findings allow for some speculation as to the role of anthropological factors, inflammation, and CVD in previous centuries. For example, a moderate level of atherosclerosis including the presence of fatty streaks, was observed in the aorta and coronary circulation of an ice-preserved 53-year-old Eskimo woman from 430 AD.⁹⁸ Interestingly, Eskimo women were responsible for trimming seal-oil lamps

throughout the night, likely resulting in substantial exposure to smoke and the development of anthracitic lungs; potentially analogous to the effects attributed to cigarette smoking in today's society.⁹⁸ In ancient Egypt, tomb reliefs describe sudden deaths that are believed to be attributable to myocardial infarction and cerebrovascular events.⁹⁸ Accordingly, the presence of atherosclerosis was found in both large and small arterial segments from mummies preserved more than 3000 years.⁹⁸ Recently, lesions collected in the mid 19th century were found to possess CD3-positive cells when in early stage disease, consistent with a role of inflammation in atherogenesis.³ Thus, it would appear as though the progression and phenotype of atherosclerotic disease has remained similar throughout the last several thousand years. Although virtually impossible to directly correlate inflammation and CVD throughout history, enhanced exposure to inflammation-promoting factors and greatly increased life expectancy in modern times may account for the current epidemic nature of this disease. It is also important to consider that CVD prevalence in today's Western populations has occurred in the face of tremendous advancements in the fields of medicine in the last century.

Perspectives: Genomics to Therapeutics

Although it is unlikely that gross anatomical alterations in the vasculature or changes in the inherent immunological response of humans have occurred throughout the last 10,000 years, several lines of evidence suggest that rapid changes endured by humans throughout this time period have contributed to the increased prevalence of CVD in modern society. Furthermore, these factors have been amplified by each of the advances in sanitation, nutrition, medicine, and technology, which have contributed to the increased lifespan of contemporary humans. Reconciliation of a historical perspective with current knowledge of cellular and molecular mechanisms of disease allows us to reason that diet and other anthropolog-

Figure 3. Anthropological stimuli including nutritional characteristics, active versus sedentary lifestyles, socioeconomic class, and others dictate the expression profiles of our genome on two major levels. Chronically, these stimuli influence the widespread expression of genes, including the incorporation of beneficial mutations and the purging of undesirable genetic code. Acutely, these stimuli provoke a response that manifests as the genetic expression of an individual. Recent historical evidence demonstrates that changes in these stimuli have resulted in an amplified inflammatory response that collectively conditions and predisposes the vessel wall to plaque formation. Elucidation of these pathways at the genetic surface of populations, as well as individuals, will uncover multiple avenues of enhancing therapeutics, including risk stratification, lifestyle modifications, and personalized medicine, as well as the identification of inflammatory markers and molecular targets.

ically relevant factors drive the inflammatory process, resulting in pathologies such as atherosclerosis in Western populations. Consistent with this notion, populations that simulate hunter-gatherer lifestyles have been shown to be less susceptible to CVD.⁹⁹ Although the presentation of risk factors such as diet-related obesity, smoking, and reduced physical activity is not novel, appreciation for the central role of inflammation in the manifestation of these factors is an important concept for future therapeutics. To this end, inflammatory pathways encoded in our genes possess both the antecedent of atherogenesis and the fundamental resolutions of vascular disease (Figure 3).

Now that we have evolved into the postgenomic era, it is clear that advances in genomic techniques hold promise for transforming the practice of medicine, perhaps to personalized medicine.¹⁰⁰ The importance of this type of approach is highlighted by the propensity of individuals or cohorts to display unique patterns in terms of their responsiveness to the anthropologically relevant stimuli discussed herein. For example, data from the International Hap Map project and the Human Genome Project provide technological advances in the determination of susceptibility to lesion formation and its potential clinical consequences. Undoubtedly, small molecule profiling and high-throughput gene expression and genotyping will better characterize the pathology of the atheroma and refine the future practice of medicine. We believe that advances in genomic, proteomic, and metabolomic research will aid in the prediction of atherosclerosis and bridge basic science with epidemiology. Indeed, profiling of genes and proteins has already provided potential targets for therapeutic intervention.

A relatively novel technique known as transcriptional profiling has evolved into an effective approach for detecting nutritional (eg, fatty acids), inflammatory, and other environmental agents (viruses, pollutants) relevant to the promotion of atherogenesis.^{101,102} For example, oxidants in the diet, vessel wall shear stress, and modified LDL can now be monitored to assess their roles in vascular EC modification and altered gene expression in the vessel wall over time.¹⁰⁰ Mechanisms associated with coronary thrombosis can also be defined via platelet profiling in patients with acute coronary syndromes. In this manner, one can monitor long-term changes in gene transcription from megakaryocytes, with a focus on the differences before and after the onset of symptoms. Such an approach has identified myeloid-related proteins such as CD69 and myeloid-related protein-14 as novel markers of coronary risk.¹⁰³

Information from profiling experiments can additionally aid in the development of new cardiovascular therapeutics. Alterations in the expression profiles of genes in normal and atherosclerotic tissues have been reported in monocytic cell lines, and inflammatory genes have been found to be elevated during CVD.¹⁰⁴ Assessment of inflammatory markers, once identified from gene transcriptional profiling, can improve risk stratification.¹⁰⁰ In addition, profiling can identify drug targets and assist in the validation of candidate drugs for patient management.¹⁰⁰ For example, the target of statins, 3-hydroxy-3-methyl-

glutaryl-CoA (HMG-CoA) reductase, has been shown to increase during atherogenesis. Others have demonstrated that statins inhibit the expression of inflammatory cytokines such as IL-1 β .¹⁰⁵ Undoubtedly, the use of genomic techniques to identify genes whose expression is increased will enhance the potential to identify other drug targets associated with inflammation. Additional links identified by expression profiling have been described for carbohydrates and their associated metabolic enzymes leading to matrix modification and tissue remodeling of the plaque.¹⁰⁶ Similarly, associations have been identified for apoptosis of SMCs in the plaque involving growth arrest DNA damage (GADD) genes.¹⁰⁷ We believe that continued research and subsequent validation of genetic approaches, particularly those that elucidate and identify the inflammatory conundrum will allow for powerful advances in targeted therapeutic applications for the treatment of atherosclerosis and other CVDs.

Acknowledgments

We thank Drs. Katherine Hajjar, Aaron Marcus, and Ralph Nachman for their critical review of this manuscript.

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