The American Journal of Pathology, Vol. 182, No. 5, May 2013

The American Journal of PATHOLOGY

ajp.amjpathol.org

MINI-REVIEW

Biological Relevance of Inflammation and Oxidative Stress in the Pathogenesis of Arterial Diseases

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Accepted for publication January 4, 2013.

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Over the past three decades, age-adjusted rates of cardiovascular morbidity and mortality have fallen in the United States, but the prevalence of obesity and associated metabolic disorders has risen dramatically. Recent studies have begun to unravel the complex linkages between adipose and vascular tissues that may accelerate the development of atherosclerosis in the context of obesity. Experimental models indicate that inflammation and oxidative stress, which mutually amplify each other within the vasculature and in visceral fat, are key processes that drive the initiation, progression, and subsequent rupture of the atherosclerotic lesion. Emerging research is further elucidating the contributions made by chemokines and their receptors, adipokines, and miRNAs to arterial disease. Translation of these basic science findings to clinical applications represents a tantalizing possibility for reducing the global burden of obesity-associated atherosclerosis and other cardiovascular diseases. *(Am J Pathol 2013, 182: 1474–1481; http://dx.doi.org/10.1016/j.ajpath.2013.01.010)*

A growing body of basic and clinical evidence indicates that vascular inflammation plays a mediating role at all stages in the genesis of arterial disease. Experimental studies in animals have helped elucidate the pathophysiological inflammatory processes underlying atherosclerotic plaque development and thrombosis. In addition, the clinical validation of the acute-phase reactant C-reactive protein (CRP) as a biomarker associated with increased cardiovascular risk has lent further strength to the inflammatory hypothesis.[1,2] Inflammation can be a manifestation of increased oxidative stress, and animal studies have also provided compelling evidence to support the role of oxidative stress in atherosclerosis, particularly through oxidative modification of low-density lipoprotein (LDL).[3] Nonetheless, application of the oxidative stress model to humans remains less straightforward, given the failure of several large-scale clinical trials with antioxidants.[4] Oxidative stress does, however, remain an important pathogenic link between inflammation and atherosclerosis, particularly in the setting of obesity and associated metabolic disorders. Recent data indicate that obesity generates chronic low-grade inflammation and increased conditions of oxidative stress, both of which cause vascular perturbations that can accelerate the pace of atherosclerosis. In this Mini-Review, we provide an overview of the mechanisms linking inflammation and oxidative stress in vascular and adipose tissues to an increase in the risk for arterial disease (Figure 1). We also highlight new classes of molecules that are implicated in the inflammatory and oxidative stress responses in atherosclerosis and obesity that may participate in the communication between visceral fat and the arterial wall.

Progression of Atherosclerotic Vascular Disease

Within the arterial wall, inflammation and oxidative stress play interconnected and mutually reinforcing roles to accelerate atheroma formation. Oxidative modification of LDL particles is hypothesized to be an essential early step in the atherosclerotic process that occurs in a proinflammatory, pro-oxidant vascular milieu.[3] Circulating LDL particles are retained within the subendothelial extracellular matrix by

Supported by the NIH grants P01 HL-46403 and R01 HL-091101 and the Julia & Seymour Gross Foundation (D.P.H.).

Disclosures: D.P.H. is a consultant to KOWA Pharmaceutical Corp., Japan. A.M.G. is a consultant for AstraZeneca, Janssen, KOWA, Merck, and Roche; a member of the Board of Directors for Aegerion Pharmaceuticals and Arisaph Pharmaceuticals; and a member of advisory boards for DuPont, Haptocure, VascuVis, and Vatera Capital.

http://dx.doi.org/10.1016/j.ajpath.2013.01.010

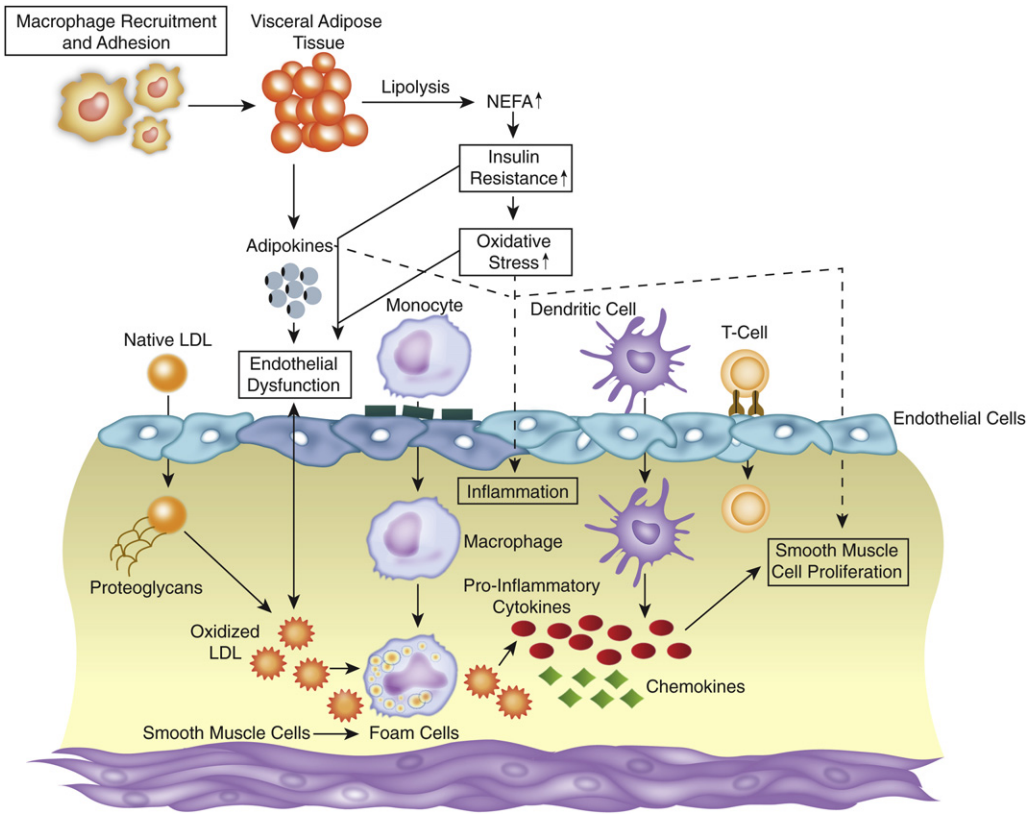

Figure 1 Mechanisms of disease in atherosclerosis and obesity. Pathophysiological processes within the vessel wall lead to the development of atherosclerosis and may be augmented by obesity-associated effects in adipose tissue. Atherosclerosis begins with the retention and oxidative modification of LDL, incorporation of oxidized LDL into burgeoning foam cells, triggering of a proinflammatory cascade, and subsequent proliferation of smooth muscle cells as the plaque progresses. Dendritic cells and T cells are drawn into the lumen by adhesion molecules and are incorporated into the atheroma. In obesity, macrophages are recruited and infiltrate adipose tissue, which can result in the release of adipokines and generation of a proinflammatory state. Under these conditions, lipolysis can lead to increased release of nonesterified fatty acids and possibly also to insulin resistance. The resulting increase in oxidative stress, combined with the action of adipokines, exacerbates the vascular pro-oxidant and proinflammatory environment, worsens endothelial dysfunction and smooth muscle cell proliferation, and accelerates the atherosclerotic process.

proteoglycans and then undergo oxidative or other chemical modifications that render them susceptible to engulfment by macrophage scavenger receptors.[5] The formation of oxidized LDL and of oxidized LDL components, such as oxidized phospholipids (OxPL), derails normal endothelial functioning. This can lead to the production of adhesion molecules on the vascular surface, including E- and P-selectin, intracellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1 (VCAM-1).[6] Furthermore, chemokines draw leukocytes, dendritic cells, and T cells from the arterial lumen into the intima, where they are later incorporated into the burgeoning atheroma. Leukocyte activation generates the enzyme and emerging biomarker myeloperoxidase which catalyzes a variety of reactive oxygen species (ROS) that may contribute to tissue damage, lipid peroxidation, and the inflammatory cycle.[7]

Oxidized phospholipids are novel biomarkers that exert mixed effects on atherosclerosis, including promotion of monocyte adhesion to endothelial cells; increased production of chemokines, proinflammatory cytokines, and growth factors; suppression of inflammation in leukocytes; and stimulation of smooth muscle cell proliferation.[8] The amount of OxPL present on apolipoprotein B-100 (OxPL/ApoB) correlates strongly with plasma levels of lipoprotein(a), which is a major carrier of OxPL in plasma.[9] Paradoxically, increases in OxPL/ApoB have been observed shortly after initiation of statin therapy, which may be due to efflux of OxPL from sites of arterial injury.[10] Phospholipase A_2 enzymes, including secretory PLA_2 ($sPLA_2$) and lipoprotein-associated phospholipase A_2 (Lp-PLA_2), degrade OxPL to produce proinflammatory and proatherogenic lipid mediators.[11] Levels of $sPLA_2$ and Lp-PLA_2 mass and activity are associated with increased cardiovascular risk and have been shown to decrease after treatment with statin therapy.[11] Inhibition of phospholipase A_2 enzymes is an experimental, anti-inflammatory approach to the treatment of atherosclerotic disease.

In the atheroma, oxidized LDL and its components activate the innate immune system by ligating Toll-like receptors. These interactions spark an intracellular signaling cascade leading to increased expression of a range of proinflammatory molecules, including cytokines, chemokines, eicosanoids, proteases, ROS, reactive nitrogen species, and costimulatory molecules.[6] Intracellular pattern-recognition

receptors form into inflammasomes, which are large multiprotein complexes that are activated by the uptake of oxidized LDL through scavenger receptors to secrete IL-1β and IL-18.[6] T-cell subtypes secrete cytokines with differential and sometimes overlapping effects: Th1 cells release proinflammatory cytokines such as interferon γ (IFN-γ); Th2 cells express cytokines, such as IL-4, that have an uncertain effect on inflammation and atherosclerosis; and regulatory T cells produce anti-inflammatory cytokines such as IL-10 and transforming growth factor β1 (TGF-β1).[1,12] Endothelial cells engulf oxidized LDL via the lectin-like oxidized LDL receptor 1 (LOX-1), which renders the cells functionally deficient.[13] The overall proinflammatory, pro-oxidant atmosphere disrupts vascular function, primarily by decreasing the bioavailability of the vasodilator nitric oxide (NO) and perpetuating conditions of oxidative stress through excess generation of ROS and reactive nitrogen species.[14] We and others believe that the effects of established cardiovascular risk factors, including smoking, dyslipidemia, hypertension, and diabetes, may additionally contribute to endothelial dysfunction, in part by unsettling the oxidative balance.[15]

Within the intimal space, ingestion of oxidized LDL by macrophages results in the formation of foam cells, the primary component of the fatty streak. Activated macrophages produce ROS and proinflammatory mediators such as IL-1β and tumor necrosis factor (TNF), further accelerating the cycle of oxidative stress and inflammation.[1] In response to chemoattractants, smooth muscle cells move into the intima and proliferate, generating matrix metalloproteinases that can digest extracellular elastin and collagen. Smooth muscle cells then encase foam cells with a fibrous cap, and foam cell apoptosis gives rise to a lipid-rich necrotic core. Proinflammatory mediators weaken the thin fibrous cap of the mature plaque, setting the stage for rupture and subsequent thrombosis.

Vascular conditions of oxidative imbalance promote atherothrombotic disease through other mechanisms besides oxidation of LDL: by modifying high-density lipoprotein (HDL) components to form a dysfunctional, proinflammatory, and pro-oxidant particle; by stimulating platelet activation; by promoting coagulation through decreased fibrinolysis and increased expression of tissue factor; and by altering local hemodynamic forces to disturb blood flow.[13] Inflammation and oxidative stress thus play multiple integral roles in the initiation, progression, and rupture of the atherosclerotic lesion.

Oxidative Stress and Inflammation in Obesity

Within vascular cells, circulating inflammatory cells and platelets, superoxide may be generated enzymatically by NADPH oxidase, myeloperoxidase, xanthine oxidase, lipoxygenases, and nitric oxide synthases, as well as by a byproduct of mitochondrial respiration.[13] Subsequent reactions involving superoxide, NO, and other free radicals can lead to ROS formation, the effects of which may be mitigated by vascular antioxidant enzymes including superoxide dismutases, catalase, glutathione peroxidases, glutathione S-transferases, heme oxygenase, and glucose-6-phosphate dehydrogenase.[13] Under conditions of oxidative imbalance, ROS generation stimulates atherogenesis (as described above). In the context of obesity and associated metabolic disturbances, the pro-oxidant shift may be even more extreme, because of overfeeding, adiposity, insulin resistance, and hyperglycemia.

A growing body of evidence indicates that obesity is accompanied by a state of chronic, low-grade, systemic inflammation that increases risk for cardiovascular disease by exacerbating the vascular inflammatory response. Circulating levels of inflammatory markers released by the liver, including CRP and serum amyloid A, are elevated in obesity and have been associated with increased cardiovascular risk.[16] Many of the pathways that lead to the production of such inflammatory mediators may be initially induced by oxidative stress. Excessive caloric intake, even before weight gain, is hypothesized to be a primary trigger of systemic inflammation and insulin resistance.[17] Studies suggest that a high metabolic load of carbohydrates and/or fats from as little as one meal can overload cells and causes excessive mitochondrial oxidation, resulting in increased production of ROS and other markers of oxidative stress.[17–19] The superoxide radical generated with overfeeding activates at least two major proinflammatory transcription factors, nuclear factor κB (NF-κB) and activator protein 1 (AP-1), and it can also induce endothelial dysfunction by decreasing NO bioavailability.[17] Reduced macronutrient intake in obese individuals is associated with decreased levels of oxidative stress and inflammatory mediators, as are food combinations incorporating sufficient fiber, fruit, and vegetables. We interpret these findings to indicate that the rapid evolution of the Western diet over the past 200 years, combined with increasingly sedentary lifestyles, a rise in cigarette smoking, and various other socioeconomic factors are major contributors to the increased prevalence of atherosclerotic vascular disease.[20]

Accumulation of visceral fat leads to adverse local and systemic metabolic effects that collectively increase oxidative stress and inflammation. In a proinflammatory obese state, lipolysis of adipose tissue is accelerated as adipocytes become locally insulin-resistant, resulting in increased levels of nonesterified fatty acids.[21] High concentrations of nonesterified fatty acids may subsequently increase ROS generation in mononuclear cells and induce insulin resistance in peripheral cells.[21] It is hypothesized that, as adipose tissue enlarges, adipocytes undergo hypertrophy and possibly hyperplasia. This can lead to ROS production and the dysfunctional secretion of a variety of proinflammatory and prothrombotic mediators, including specialized cytokines and proteins referred to as adipokines.[22] In combination with other factors, adipokines and chemoattractant cytokines such as monocyte chemotactic protein 1 (MCP-1) can activate circulating monocytes, cause them to adhere to

endothelial cells, and then draw them into adipose tissue, where they differentiate into macrophages.[23,24]

Macrophage recruitment and infiltration in adipose tissue may contribute to atherosclerosis through enhanced production of proinflammatory molecules, including TNF-α and IL-6.[25] Adipokines, some of which may be secreted by recruited macrophages, can cause a variety of proatherogenic vascular disturbances, such as inflammation, endothelial dysfunction, and smooth muscle cell proliferation, as discussed below.[22] In addition, oxidized LDL generated in adipose tissue can heighten adipocyte hypertrophy and/or hyperplasia, leading to increased hypoxia, oxidative stress, and subsequent apoptosis of adipocytes.[24] Macrophages accumulate predominantly around areas of dead adipocytes, but oxidized LDL inhibits their normal function of phagocytic removal. Support for the involvement of oxidized LDL in adipose tissue is suggested by studies linking obesity and the metabolic syndrome with elevated levels of circulating oxidized LDL.[26]

Obesity and the metabolic syndrome can lead to the development of insulin resistance and type 2 diabetes. Insulin resistance exacerbates oxidative stress in part by increasing the mitochondrial production of ROS from nonesterified fatty acids, increasing ROS production from other sources, and inactivating antioxidant enzymes.[27] Hyperglycemia induces ROS generation through multiple pathways and can have direct, deleterious effects on endothelial function.[28] Hyperglycemia-induced production of ROS leads to increased levels of advanced glycation end products (AGEs) and their receptor (RAGE), both of which can activate pathways leading to further production of ROS.[27] Binding of AGEs to RAGE triggers ROS generation and subsequent activation of NF-κB, causing increased expression of ICAM-1, VCAM-1, and the procoagulant proteins plasminogen activator inhibitor 1 (PAI-1) and tissue factor.[28] Uptake of AGEs by macrophage receptors also accelerates atherosclerosis by promoting inflammation and smooth muscle cell proliferation, whereas its uptake by endothelial cells has vasoconstrictive and prothrombotic effects.[27,28] Additional stress in diabetes arises from a decrease in HDL cholesterol levels and an increase in the oxidized fatty acid content of HDL, which impairs the protective, antioxidant, and anti-inflammatory capacity of the particle.[29] Under conditions of oxidative stress and inflammation, derangement of normal HDL functioning can lead to increased oxidation of LDL, interference with NO-mediated endothelial dilation, and impairment of reverse cholesterol transport.[30]

Emerging Biomarkers and Therapeutic Targets

Although experimental studies indicate that oxidative stress and inflammation play mutually exacerbating roles within the vasculature and adipose tissue that may contribute to an accelerated atherosclerotic disease process, the therapeutic use of antioxidants has not shown clinical benefit in humans.[4] Past trials, which tested primarily vitamin E and β-carotene, were likely unsuccessful because of an incomplete understanding of the mechanisms behind LDL oxidation, which may have led to the use of the wrong antioxidant, insufficient strength and/or duration of dosing, and inappropriate patient selection.[3,31] The antioxidants may also have been unable to localize at vulnerable sites within the arterial wall. One novel class of antioxidants currently under development includes small peptide molecules targeted at the inner mitochondrial membrane, which have shown promise for the potential treatment of the mitochondrial dysfunction that underlies many cardiorenal, neurological, and metabolic disorders.[32] We believe that additional basic research is necessary to clarify the specific site of LDL oxidation, the fate of oxidized LDL *in vivo*, and the exact contribution of obesity-associated oxidative stress to the atherosclerotic process. At the same time, newly identified classes of molecules that mediate inflammation and oxidative stress within the vessel wall and in adipose tissue are emerging as potential biomarkers and therapeutic targets for atherosclerotic vascular disease.

Chemokines and Chemokine Receptors

Chemokines participate in atherosclerotic plaque development by recruiting leukocytes (including neutrophils, monocytes, T and B cells, dendritic cells, and mast cells) from the lumen into the subendothelial space at sites of inflammation.[33] Chemokines may be fixed on the surface of endothelial cells by an array of proteoglycans and can activate G protein coupled receptors to produce leukocyte integrins. Alternatively, soluble chemokines may steer leukocytes into the arterial wall directly. Individual chemokines have corresponding receptors, but may bind to multiple receptors, a characteristic referred to as redundancy. Most chemokines and their receptors promote inflammation, although some are thought to be atheroprotective. They also vary in the types of atherogenic mononuclear cells recruited, the sites of lesion formation to which leukocytes are drawn, and the stages of lesion formation in which they are primarily involved.[34]

Chemokines or chemokine receptors that play an established role in atherosclerosis include CXCR3 and its ligands, which recruit effector T cells to plaques; CCR2 and its ligand CCL2 (alias MCP-1) and CX3CR1 and its ligand CX3CL1, which draw monocytes to plaques; and CCR5 and its ligand CCL5, which attract both T cells and monocytes.[34] Studies in mice have shown that genetic deletion of these chemokines or their receptors protects against atherosclerotic lesion formation and, in some cases, decreases neointimal hyperplasia. In animal models of injury, elevated circulating levels of CCL2 and CXCL10 have been detected early in the heart, suggesting that chemokines may play a role in leukocyte recruitment during the postinfarction inflammatory response.[35] Novel chemokines have also been identified that may recruit neutrophils and monocytes from bone marrow, promote monocyte survival within the atherosclerotic

plaque, control the expansion of regulatory T cells in plaques and lymph nodes, and prime T cells before drawing them out from lymph nodes.[36] It has recently been recognized that chemokine receptors (CXCR2, CCR2, and CXCR4) are present on cardiac myocytes and are up-regulated after oxidative stress; these receptors may play a role in ischemia−reperfusion injury and in the pathophysiology of heart failure.[35]

The chemokine system offers numerous potential targets to reduce atherosclerotic inflammation, and the CCL2−CCR2 pathway has been a particular site of investigation. CCL2 (MCP-1) is synthesized by smooth muscle cells, endothelial cells, and macrophages in response to lipopolysaccharide and TNF, and exposure of endothelial cells to oxidized LDL strongly up-regulates its production.[37] Small-molecule inhibitors of the CCL2−CCR2 axis have shown mixed results in animal models, although a monoclonal antibody (MLN1202) to CCR2 was shown to reduce levels of CRP independent of statin use in a small phase 2 trial.[36,37] Additional research is necessary to determine the mechanisms behind this effect, the anatomical location of the targeted monocytes and macrophages, and the clinical implications of this approach. Novel classes of chemokine blockers currently under development include antagonists generated through structural modification of chemokines and chemokine-neutralizing proteins isolated from pathogens, such as the evasins recently discovered in tick saliva.[36] In addition, stem cell therapy approaches to repair infarcted cardiac tissue by targeting the CXCR4 receptor and its associated chemokine CXCL12 are under investigation.[35]

Adipokines

Adipokines are hypothesized to act as a bridge of communication between adipose and vascular tissues, thereby linking obesity with increased risk for cardiovascular disease. Most adipokines induce proinflammatory processes in the vasculature, particularly by activating NF-κB signaling, and their expression is increased in obesity.[22] An important exception is adiponectin, which is decreased in obese adipose tissue, and has demonstrated insulin-sensitizing, anti-inflammatory, and antiproliferative properties. Activation of NF-κB by proinflammatory adipokines may increase the expression of adhesion molecules ICAM-1, VCAM-1, and E-selectin, thus promoting the recruitment of monocytes into the vessel wall. NF-κB activation can also contribute to the proliferation of intimal smooth muscle cells and can mediate the expression of proinflammatory molecules by macrophages and smooth muscle cells.

The evidence linking adipokines with cardiovascular disease is strongest for adiponectin and leptin. Adiponectin, which is considered to be cardioprotective, decreases the vascular inflammatory response by suppressing TNF-α and IL-6. This improves endothelial function by increasing NO production; it also reduces smooth muscle cell proliferation and migration, and has been associated with improvements in the lipid profile.[22] Adiponectin levels are reduced both in animal models and in patients with obesity and insulin resistance, possibly because of neuroendocrine alterations and increased conditions of oxidative stress and inflammation.[16,38] Leptin, which decreases appetite, is thought to be proatherogenic, although studies linking circulating levels of leptin with cardiovascular disease have been inconclusive.[22] The vascular effects of leptin include activation of endothelial NO synthase (eNOS) to increase NO production in endothelial cells, increased expression and activity of inducible NO synthase (iNOS) by smooth muscle cells, and increased expression of PAI-1 and CRP in endothelial cells. Leptin may increase oxidative stress through multiple mechanisms and may contribute to the pathogenesis of insulin resistance and hypertension as well as atherosclerotic disease.[39]

The adipokines TNF-α and migration inhibitor factor (MIF) are macrophage-associated proinflammatory cytokines that correlate with increased cardiovascular risk.[22] Both may induce migration and proliferation of smooth muscle cells, and TNF-α additionally promotes endothelial dysfunction, insulin resistance, and lipolysis in adipocytes.[16,22] Expression of MIF, which is produced in all cell types present in atherosclerotic plaques, is triggered by oxidized LDL and is up-regulated as lesions develop.[40]

Small lipid-binding proteins, including adipocyte-type fatty acid−binding protein (A-FAPB), lipocalin-2, and retinol binding protein 4 (RBP-4) are a class of adipokines that transport lipophilic substances to facilitate their metabolic processing or to sequester them within the cell.[41] Although their physiological functions in adipose tissue and the vasculature are largely uncharacterized, levels of A-FABP and lipocalin-2 are elevated in patients with obesity, metabolic syndrome, and coronary heart disease, and these adipokines have been correlated with various markers of increased cardiovascular risk, including lipoproteins, adiposity, inflammation, endothelial dysfunction, blood pressure, and insulin resistance.

This is a very interesting set of observations. Studies in genetically modified mice now indicate that lipocalin-2 deficiency protects against diet-induced elevations in systolic blood pressure and endothelial dysfunction, whereas administration of lipocalin-2 attenuated endothelium-dependent relaxations and instead promoted contractions.[42] Similarly, deletion of the A-FABP gene protects against obesity-associated insulin resistance and reduces inflammation and atherosclerotic lesion development in *ApoE*$^{-/-}$ mice.[43] Serum levels of A-FABP correlate with levels of lipocalin-2, and expression of lipocalin-2 is induced by proinflammatory stimuli including lipopolysaccharide, IL-17, TNF-α, hyperglycemia, and IL-1β through activation of NF-κB.[41] The effects of the lipid-binding protein RBP-4 appear to be related mainly to insulin resistance.[16] Inhibitors of A-FABP have shown beneficial effects in rodent models, including reductions in foam cell formation, proinflammatory cytokines, and atherosclerotic lesion development, as well as improvements in endothelial function and insulin sensitivity.[41]

A variety of proinflammatory interleukins are secreted by adipose tissue, including IL-1β, IL-8, and IL-18, and these have been demonstrated to have atherogenic effects on vascular smooth muscle and endothelial cells, in addition to inducing the production of other cytokines and chemokines.[22] A large clinical trial investigating the effects of IL-1β inhibition with the monoclonal antibody canakinumab on post-myocardial infarction patients with elevated CRP levels is currently underway.[44] The anti-inflammatory adipokine IL-10 is thought to have antiatherogenic actions, including inhibition of IL-6 and IL-8 production in endothelial cells, partial inactivation of NF-κB leading to reduced smooth muscle cell infiltration, and increased NO production.[22] An upcoming clinical trial is designed to assess the effects of methotrexate, an anti-inflammatory agent that reduces levels of IL-6, TNF-α, and CRP, on cardiovascular events in patients with stable coronary artery disease (*http://clinicaltrials.gov/ct2/show/NCT01594333*, last accessed March 1, 2013).[1]

Novel adipokines that have less clear associations with the development of atherosclerosis include chemerin, resistin, visfatin, vaspin, and omentin.[16,22] Studies suggest that these adipokines may be elevated in obesity, could function as inflammatory mediators, and may modulate insulin sensitivity; however, these adipokines currently lack knockout or transgenic mouse studies, data are sometimes contradictory, and their relevance in humans remains to be clarified. Adipokines have been implicated in multiple disease processes other than obesity and cardiovascular disease. We believe that ongoing research may help elucidate the mechanisms by which adipokines can affect multiple organs and tissues. We hope that additional data will be forthcoming regarding the viability of adipokines as potential therapeutic targets for obesity-associated atherosclerotic disease, as others have suggested.[45]

miRNAs

miRNAs are abundant in many different cell types, with recognized contribution toward many biological processes. miRNAs are single-stranded, noncoding RNAs of approximately 22 nucleotides that act post-transcriptionally on gene expression by binding to mRNA target sequences to regulate protein translation or mRNA stability.[46] miRNAs may have hundreds of mRNA targets, and are thought to exert a relatively modest, nuanced effect on mRNA expression, compared with transcription factors, and some mRNA targets may be modulated by multiple miRNAs.[47] miRNAs have been associated with various biological processes that may contribute to the development of atherosclerosis, type 2 diabetes, and cardiovascular disease. Recent studies indicate that miR-33 isoforms play a role in lipid metabolism by down-regulating transporters involved in cholesterol efflux; however, the inhibition of miR-33 in mice caused an increase in cholesterol efflux and circulating HDL levels.[48] Antisense oligonucleotides to miR-33 are being investigated for the treatment of cardiometabolic disorders, after studies in nonhuman primates demonstrated increases in HDL and decreases in triglycerides with antisense inhibition.[47] Given the current state of knowledge, it appears that therapeutic targeting of miR-33 to modulate cholesterol and lipid homeostasis is one of the most promising areas of investigation in the field of miRNA research.

A number of miRNAs are also thought to participate in atherogenesis by modulating the vascular inflammatory response, production of adhesion molecules, monocyte differentiation and uptake of oxidized LDL, and smooth muscle cell proliferation.[46,49] For example, miR-21 has been shown to repress superoxide dismutase-2, resulting in increased ROS production and impaired availability of NO.[50] In obese adipose tissue, miRNAs are hypothesized to regulate adipocyte differentiation, oxidative stress, inflammation, and angiogenesis.[46] Preliminary studies indicate that several miRNAs may play a role in obesity-associated insulin resistance by influencing insulin signaling and glucose homeostasis.[47] For example, miR-223 has been shown to control macrophage activation in adipose tissue, resulting in an attenuation of diet-induced inflammation and systemic insulin resistance.[51] It is hypothesized that the pathogenic processes that occur in obese adipose tissue and in the vasculature may be linked by several miRNAs that display similar functions in both tissue types, including regulation of cell proliferation, angiogenesis, apoptosis, and inflammation.[46] Additionally, adipocytes have been found to secrete microvesicles that contain miRNAs; intercellular communication between adipose and vascular tissues could potentially occur through this route.

A major challenge facing the development of miRNA-based therapeutics is the lack of specificity of individual miRNAs to metabolic processes, given their relatively modest effects on mRNA expression and potentially overlapping actions on multiple mRNA molecules at once. miRNAs that mediate pathological processes in both vascular and adipose tissue may be particularly useful targets for the treatment of obesity-associated vascular diseases.[46] Nonetheless, the development of miRNA-based therapeutics remains primarily at the preclinical stage, and challenges regarding target identification, specificity, mode of delivery, and length of action need to be overcome before testing in clinical trials.[48]

Conclusion

In the arterial wall, increases in inflammation and oxidative stress synergize to accelerate atheroma formation and increase risk for arterial disease. Studies in recent years have generated a clearer understanding of how obesity-associated inflammation and oxidative stress could be implicated in other pathophysiological processes, including endothelial dysfunction, macrophage recruitment and adhesion, smooth muscle cell proliferation, and insulin resistance, all of which further contribute to atherosclerotic plaque development. New classes of molecules that link obesity and

atherosclerosis, and inflammation with oxidative stress have emerged as potential biomarkers and therapeutic targets. Further research on chemokines and their receptors, adipokines, and miRNAs could, we believe, lead to new strategies for intervention in the prevention and treatment of obesity-associated atherosclerosis and other cardiovascular diseases.

Acknowledgment

We thank Dr. Jennifer E. Moon for her assistance with the preparation of this manuscript.

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