The Role of Periadventitial Fat in Atherosclerosis
An Adipose Subset With Potential Diagnostic and Therapeutic Implications

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Context.—It has become increasingly evident that adipose tissue is a multifunctional organ that produces and secretes multiple paracrine and endocrine factors. Research into obesity, insulin resistance, and diabetes has identified a proinflammatory state associated with obesity. Substantial differences between subcutaneous and omental fat have been noted, including the fact that omental fat produces relatively more inflammatory cytokines. Periadventitial fat, as a specific adipose tissue subset, has been overlooked in the field of atherosclerosis despite its potential diagnostic and therapeutic implications.

Objective.—To review (1) evidence for the role of adventitial and periadventitial fat in vessel remodeling after injury, (2) the relationship between adventitial inflammation and atherosclerosis, (3) the association between periadventitial fat and plaque inflammation, and (4) the diagnostic and therapeutic implications of these roles and relationships for the progression of atherosclerosis.

Data Sources.—We present new data showing greater uptake of iron, administered in the form of superparamagnetic iron oxide, in the periadventitial fat of atherosclerotic mice than in control mice. In addition, macrophage density in the periadventitial fat of lipid-rich plaques is increased compared with fibrocalcific plaques.

Conclusions.—There is a striking paucity of data on the relationship between the periadventitial fat of coronary arteries and atherosclerosis. Greater insight into this relationship might be instrumental in making strides into the pathophysiology, diagnosis, and treatment of coronary artery disease.

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Adipose tissue is distributed ubiquitously throughout the body. Its cellular components include mature adipocytes, preadipocytes, endothelial cells, nerve fibers, monocytes, and macrophages. It is becoming increasingly evident that adipose tissue is a multifunctional organ that produces and secretes multiple factors that can act in both paracrine and endocrine fashion. These factors cover a wide spectrum and include peptides, proteins, prostaglandins, and hormones. It should be noted that not all substances produced or secreted by adipose tissue are necessarily produced by adipocytes. Adipocyte-secreted cytokines, such as tumor necrosis factor α, interleukin 6, and macrophage migration inhibitory factor, are known to be produced by macrophages. Macrophage migration inhibitory factor has even been proposed to be a molecular link between adipocytes and macrophages. Our group has recently shown that adipocytes produce C-reactive protein (CRP) in culture when stimulated by appropriate cytokines.

Research in the fields of endocrinology, obesity, insulin resistance, and diabetes has identified a proinflammatory state associated with increased adiposity. Adipocytes grow in response to increased fatty acid availability, and as they become larger, their cellular homeostatic and secretory profiles evolve toward a more inflammatory phenotype. This change is accompanied by the activation of circulating mononuclear cells. Although macrophages and adipocytes derive from different lineages (hematopoietic and mesenchymal, respectively), the gene expression profile of fat cells from obese individuals resembles that of macrophages. Insulin resistance and the metabolic syndrome have been linked specifically with increased abdominal, as opposed to subcutaneous, obesity.

Multiple studies have described substantial differences between subcutaneous and intra-abdominal fat. It has been noted that adiposity significantly correlates with the expression of inflammatory cytokines in abdominal subcutaneous tissue and that omental fat contains more monocytes and macrophages than does subcutaneous fat, although this difference is not always very marked. The latter observation may be of importance to the fields of obesity and insulin resistance. The omentum is more
metabolically active and contains more blood vessels and sympathetic nerve fibers than does its subcutaneous counterpart. Omental fat cells secrete more interleukin-6 and plasminogen activator inhibitor-1 than subcutaneous cells. In addition, visceral and subcutaneous fat exhibit differential gene expression patterns in both fasting and nonfasting states.

Recently, it has become clear that proinflammatory cytokines in hypertrophied adipose tissue contribute to cardiovascular disease. Inflammatory mediators are known to be involved in the pathogenesis of coronary artery disease, and the adipose tissue is a major source of these cytokines. This suggests that periadventitial fat, as a subset within the visceral fat, has been overlooked for its potential diagnostic and therapeutic value. The present review will discuss (1) available evidence for the possible role of the adventitia and periadventitia in restenosis following vascular injury; (2) the relationship between adventitial inflammation and atherosclerosis, especially in light of the fact that inflammatory cells may not be restricted to the adventitia, but may infiltrate periadventitial fat as well; (3) the available, although scarce, evidence for a link between periadventitial fat and atherosclerosis and for its possible role in the progression and complications of atherosclerosis; and (4) the potential diagnostic and therapeutic implications of these findings.

ADVENTITIA, PERIADVENTITIA, AND VASCULAR INJURY

The adventitial response to injury has long been considered to be critical to the process of constrictive vascular remodeling that leads to restenosis following vascular injury. In porcine models of coronary artery balloon injury, it has been shown that adventitial myofibroblasts proliferate, synthesize growth factors, and migrate to the intima, where they induce intimal hyperplasia. Increased synthesis of α-smooth muscle actin in the adventitia is believed to be an important factor in the remodeling process because cells proliferate more rapidly in the adventitia than in the media after angioplasty. In that case, the protective effect of radiation on intimal hyperplasia after balloon injury appears to be the result of inhibition of adventitial cell proliferation.

In a porcine model of coronary artery balloon angioplasty, Okamoto et al showed that the inflammatory response not only involved the entire perivascular space but also extended several millimeters beyond the vessel wall itself. The authors hypothesized that the perivascular reaction plays a key role in vascular remodeling and restenosis by fostering the recruitment and proliferation of myofibroblasts. Evidence in support of this notion has come from microscopic computed tomographic scans of stented porcine coronary arteries showing massive but disorganized arrays of vessels extending to the periadventitial region.

In swine, adventitial (vasa vasorum) angiogenesis occurs within 3 days after experimental coronary angioplasty, and regression of adventitial vessels correlates with arterial narrowing, suggesting that the adventitial microvasculature greatly influences arterial remodeling. Barker et al demonstrated this influence by occluding the adventitial vasa vasorum in pigs and later observing intimal smooth muscle-rich proliferating lesions, which suggested a role for arterial hypoxia in the initiation of intimal hyperplasia.

The literature contains only indirect evidence of the presence of macrophages in the periadventitia. In a study in which intimal thickening was induced in rabbit carotid arteries by encircling them with a silicone collar, De Meyer and associates reported the expression of inducible nitric oxide synthase by macrophages and T lymphocytes in the periadventitial granulation tissue of the arteries. Others showed that P-selectin, a potent recruiter of leukocytes, induced adventitial inflammation and vascular shrinkage in a balloon-injury model.

The therapeutic potential of blocking macrophage infiltration of the adventitia was highlighted by Danenberg et al, who showed that macrophage depletion (achieved with clodronate-containing liposomes) reduced neointimal formation in hypercholesterolemic rabbits and rats after mechanical injury. In control animals, by comparison, macrophages not only infiltrated the adventitia but also extended into the periadventitial fat. The authors concluded that this blockade prevented the macrophages from influencing smooth muscle cell migration and proliferation.

ADVENTITIA AND ATHEROSCLEROSIS

There is evidence in the literature for the role of adventitia in atherosclerosis. In experimental models of atherosclerosis, angiogenic vessels proliferate markedly and early in both the adventitia and the intimal plaque. Transfection of adventitial fibroblasts with recombinant endothelial nitric oxide synthase has been shown to induce the relaxation of endothelium-denuded segments of artery.

In studying the localization of toll-like receptor 4–positive cells in atherosclerotic human coronary arteries, Vink et al located such cells in both the plaque and adventitia. Toll-like receptor 4 is a pattern-recognition receptor involved in the innate immune response to various microorganisms and to other exogenous and endogenous stress factors potentially involved in the pathogenesis of atherosclerosis, such as lipopolysaccharides and heat-shock proteins. Interestingly, the toll-like receptor 4–positive cells in the adventitia included both macrophages and fibroblasts. Exposure of these fibroblasts to lipopolysaccharides led to activation of nuclear factor κ B and to increases in the levels of several inflammatory cytokines.

Kohchi and coworkers described adventitial inflammation, often in association with nerve involvement, in the adventitia of coronary arteries of patients with angina. The authors hypothesized that their findings might be related to the vasospastic component of unstable angina. In studying nonintimal factors associated with plaque disruption, Moreno and associates found that adventitial inflammation, along with other changes in the interface between intima and media (e.g., media inflammation, atrophy, and fibrosis), occurred more often in disrupted plaques than in stable plaques. The authors also noted that, in patients with American Heart Association class III lesions, mild adventitial inflammation increased with disease progression. Similar findings have been reported by others.

Adventitial inflammation has been implicated in unstable angina and even in intimal neovascular proliferation. The complexity and frequent organization of the inflammatory infiltrate in advanced lesions suggest the local generation of humoral immune responses in which B and T lymphocytes are critical. Other vessels may be involved in chronic inflammation, often in association with nerve involvement, in the adventitia of coronary arteries of patients with angina. The authors hypothesized that their findings might be related to the vasospastic component of unstable angina. In studying nonintimal factors associated with plaque disruption, Moreno and associates found that adventitial inflammation, along with other changes in the interface between intima and media (e.g., media inflammation, atrophy, and fibrosis), occurred more often in disrupted plaques than in stable plaques. The authors also noted that, in patients with American Heart Association class III lesions, mild adventitial inflammation increased with disease progression. Similar findings have been reported by others.
lymphocytes outnumber T lymphocytes, similar to responses seen in mucosa-associated lymphoid tissue. Therefore, it is likely that the immunologic reaction develops in response to antigens released during a long-standing process of tissue injury and inflammation.

Interest has also been paid to mast cells, whose numbers are increased in culprit arteries and also (in degranulated form) in segments containing ruptured plaques. At least 1 group has described the neurogenically stimulated release of vasoactive compounds by mast cells, especially in advanced coronary lesions. Proliferation of vasa vasorum into the intima may make plaques more vulnerable to rupture and inflammation. This was confirmed by Higuchi and coworkers, who also noted a greater density of lymphocytes in the adventitia of culprit plaques than in their intima. These findings are not surprising in light of the total cross-sectional area of the vasa vasorum in the adventitia.

The adventitia is the site of parasympathetic innervation of the coronary arteries. Acetylcholine released by these terminals diffuses through the media, reaching the endothelium to activate its muscarinic receptors and release nitric oxide.

The outer layers of the coronary artery (the media and particularly the adventitia) are vulnerable to diabetes-induced redox-sensitive injury, leading to increased inflammatory gene expression, adventitial release of cytokines and chemokines, homing of blood-borne inflammatory cells into affected segments of the vessel wall, cellular cross talk, and development of intimal lesions. Intercellular adhesion molecule 1 is highly expressed in adventitial vasa vasorum and thus constitute an important vascular port of entry for inflammatory cells. In reviewing the role of the adventitia in vascular function, Gutterman concluded that adventitial fibroblasts may actively participate in preatherosclerotic subintimal proliferation, give rise to calcium deposition in chronic atherosclerotic lesions, and help transmit neurogenic signals when endothelial function is reduced. He also proposed that, in the future, it may be possible to manage and treat coronary atherosclerosis by targeting gene or drug therapies to the adventitia.

PERIADVENTITIAL FAT AND ATHEROSCLEROSIS

Periadventitial fat has been neglected in the field of atherosclerosis, even though the vessels most prone to serious atherosclerotic complications (ie, the coronary arteries, carotid arteries, and aorta) are coated with it. This is because atherosclerosis is an intimal disease of large and medium-sized arteries, and most of the literature is devoted to the intima and to the smooth muscle cells that reach the intima from the media.

Although limited, the available data regarding periadventitial fat are intriguing. High levels of interleukin 6, a major proinflammatory cytokine that induces the production of CRP, are released by omental and subcutaneous fat in obese individuals. Subcutaneous fat expresses CRP, and chemokines, such as monocyte chemoattractant protein 1, are known to be produced by adipose tissue, raising the possibility that these proinflammatory molecules have a local effect. To date, there have been 3 reports that highlight local chronic inflammation as a potential contributing factor to the pathogenesis of coronary artery disease. These results provide further support of the association between periadventitial fat and atherosclerosis.

Mazurek et al compared the expression of monocyte chemoattractant protein 1 and inflammatory cytokines in samples of periadventitial fat from the right coronary arteries and subcutaneous fat of individuals undergoing elective coronary artery bypass grafting. All of the inflammatory mediators were expressed at much higher levels in the perivascular fat than in the subcutaneous fat, raising...
Figure 2. A. CD68 immunostaining for macrophages in human coronary arteries. Coronary section from a patient with a typical fibroatheroma showing a lipid core, a large number of plaque macrophages, and a thin cap. The inset (D) shows a large number of macrophages in the periadventitial fat. B. Stable fibrocalcific plaque. Macrophages are clearly less densely packed in the plaque and periadventitial fat. The inset (E) shows only rare macrophages in the adventitia and periadventitial fat. C. Section of a normal coronary artery. Very few macrophages are seen in the adventitia and periadventitial fat. The inset (F) shows practically no macrophages (original magnifications ×10 [A through C] and ×40 [D through F]).

Figure 3. Macrophage density in the periadventitial fat of coronary arteries containing a lipid core versus coronary arteries containing fibrocalcific plaques (P < .01).

the possibility that the perivascular inflammatory mediators might have played a significant role in the disease process. However, the authors did not correlate their findings with the clinical status of the patients or the culprit plaques. Interestingly, cytokine and chemokine levels were not affected by the use of statins or angiotensin-converting enzyme inhibitors.

In a study of similar design, Baker et al studied various adipocytokines in epicardial fat biopsies from patients who had undergone coronary artery bypass grafting using reverse transcriptase/polymerase chain reaction. Effects of certain chronic medications (eg, statins) were also assessed. Epicardial adipose tissue demonstrated much higher macrophage infiltration (by CD45 mRNA expression), and adiponectin expression was significantly lower compared with other adipose depots, a finding also supported by Iacobellis et al. Interleukin 6 mRNA expression appeared to be reduced by statins.

Phagocytic and microbicidal properties have been observed in adipose tissue. Several groups, including ours, are interested in the use of superparamagnetic iron oxide as a contrast agent for magnetic resonance imaging of atherosclerotic plaques. These nanoparticles are taken up not only by macrophages of the reticuloendothelial system and by plaque macrophages, but also by macrophages in periadventitial fat. Studies in both mice and humans support this notion. In a mouse study, we injected superparamagnetic iron oxide (Feridex I.V., 1 mmol/kg iron, which corresponds to 1.65 mg of iron per kilogram; Berlex Laboratories, Montville, NJ) intravenously into 12-month-old apolipoprotein E-deficient (atherosclerotic) mice and into 6-month-old C57BL/6 female (control) mice; the mice were killed 6 days later. When we examined perfusion-fixed serial sections of their aortas, we found prominent iron deposits in the periadventitial fat of the atherosclerotic mice, particularly inside ovoid and spindle-shaped cells that appeared to be much more numerous in the atherosclerotic samples (Figure 1). Because these cells were positive for the Mac-2 antibody, we characterized them as macrophages or macrophage-like cells. We could not, however, determine whether they had existed in the periadventitial fat before the atherosclerotic lesions had developed. To our knowledge, this is the first...
report of macrophage-like activity in periartrial fat outside the setting of percutaneous coronary intervention. Unfortunately, the fact that mice do not have a well-defined adventitial layer limits the ability to draw general conclusions from this murine model.

In light of the suggestion by Mazurek et al and Baker et al that increased inflammatory elements in the periadventitial fat, including macrophages, represent a significant pathophysiologic state that both influences and reflects the underlying tissues, we analyzed the macrophage content of the periadventitial fat of coronary arteries of patients with severe coronary artery disease in 2 different plaque phenotypes. We compared 32 arterial segments containing lipid cores (American Heart Association classification types IV and V a) against 24 arterial segments containing stable, fibrocalcific plaques (types Vb and Vc) positive for the KP-1 monoclonal antibody. We found that macrophages were more numerous and more densely packed in the periadventitial fat of atherosclerotic arteries containing large lipid cores than in the periadventitial fat of either fibrocalcific or nonatherosclerotic vessels (Figure 2), indicating a greater degree of macrophage infiltration (Figure 3). We also confirmed by negative staining for S100 or toluidine blue that these cells were indeed macrophages and not dendritic cells or mast cells, respectively. Moreover, the lack of clear boundaries between the adventitia and the immediate periadventitial fat suggested that the 2 structures were functioning as a physiologic unit. This lack of a functional border between the adventitia and periadventitial fat was noted earlier by Okamoto and coauthors in the setting of percutaneous coronary intervention, and we have expanded this observation to atherosclerotic plaque.

How can these findings be reconciled with those reported in the literature? The response-to-injury hypothesis postulates that endothelial dysfunction is the initial step in atherogenesis and that it can be induced directly by injury, including trauma caused by hemodynamic forces, and indirectly by risk factors for atherosclerosis. This hypothesis has been challenged by Hermann and associates, who found that, in hypercholesterolemic pigs, neovascularization of coronary vasa vasorum precedes endothelial dysfunction (see previous discussion). Therefore, it is possible that significant numbers of monocytes or macrophages could enter a plaque through the adventitia or periadventitial fat even in the early stages of the atherosclerotic process. The transport of significant numbers of macrophages through a markedly expanded vasa vasorum has been observed in advanced atherosclerotic disease, but no correlation with increased fat neovascularization has previously been noted. Macrophages homing via the periadventitial fat vasa vasorum (as opposed to the luminal artery) may play a significant role in atherosclerosis. Taken together, these studies raise the possibility that periadventitial fat may mediate such “outside-to-inside” signaling and thus play an important role in the development and progression of atherosclerosis and its complications.

### POTENTIAL DIAGNOSTIC AND THERAPEUTIC IMPLICATIONS OF PERIADVENTITIAL FAT

The uptake of iron by periadventitial fat macrophages suggests a potential means of imaging areas of arterial inflammation. This periadventitial uptake should improve the spatial resolution of magnetic resonance imaging—the greatest drawback of that technology—and thus allow a major advance in the noninvasive imaging of vulnerable atherosclerotic plaques. Meanwhile, new developments in molecular imaging as well as positron emission tomography and computed tomography should allow investigators to distinguish inflammatory and metabolically active adipose tissue from quiescent visceral fat.

The presence of CRP and other bioactive molecules in periadventitial fat has diagnostic potential too. C-Reactive protein is a widely used systemic marker of inflammation, and elevated high-sensitive CRP is a risk factor for coronary artery disease. High-sensitive CRP is also associated with obesity, insulin resistance, and diabetes mellitus. Adipose tissue secretes several bioactive substances (eg, leptin, high-sensitive CRP, tumor necrosis factor α, adiponectin) that have a major impact on atherosclerotic lesions. Adiponectin opposes tumor necrosis factor α and is inversely associated with high-sensitive CRP in plasma and adipose tissue. Moreover, hyperadiponectemia has been observed in obesity, diabetes, and cardiovascular disease. It is unknown whether autonomic stimulation affects periadventitial fat. Interesting data from several studies suggest a possible association between a lack of periadventitial fat and protection against atherosclerosis. For reasons that are still not clear, atherosclerosis appears to be suppressed in intramyocardial segments of coronary arteries (Figure 4).

Hemodynamic factors are usually implicated. Likewise, the intramural aortic segments of anomalous coronary arteries arising from a wrong sinus of Valsalva also appear to resist atherosclerosis. In both instances, it seems plau-
sible to attribute the absence of atherosclerosis to the lack of periadventitial fat.

The potential involvement of periadventitial fat in atherosclerosis has certain implications for therapeutic delivery methods. Pericardial drug delivery is most promising because the entire coronary tree and its surrounding fat are in direct contact with the overlying pericardial fluid. Perivascular intrapericardial delivery of nitric oxide donors has been successfully used in experimental animals to prevent restenosis after vessel injury.\(^64,65\) It is unknown, however, whether such therapy primarily affects the periadventitial fat, the adventitia, or both. Other therapeutic compounds that have been delivered experimentally via the intrapericardial route include fibroblast growth factor-2,\(^66\) procainamide,\(^67\) nitroglycerin,\(^68\) and heparin.\(^69\)

Gene therapy techniques have been used to transfer protein-encoding DNA of proteins into vascular tissue.\(^70\) In one experimental study in rats, replication-deficient adenoviral vectors containing the β-galactosidase gene were injected into the intracerebroventricular region and successfully transfected adventitial cells in the subarachnoid space.\(^71\) So far, other genes transferred into vascular cells include genes for endothelial nitric oxide synthase,\(^35\) superoxide dismutase,\(^72,73\) and angiogenic factors.\(^74,75\) Application of gene therapy techniques to the periadrenal space could have a major impact on the treatment of coronary artery disease.\(^76\)

References


