Proinflammatory Phenotype of Perivascular Adipocytes

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Abstract—Perivascular adipose tissue (PVAT) directly abuts the lamina adventitia of conduit arteries and actively communicates with the vessel wall to regulate vascular function and inflammation. Mounting evidence suggests that the biological activities of PVAT are governed by perivascular adipocytes, a unique class of adipocyte with distinct molecular and phenotypic characteristics. Perivascular adipocytes surrounding human coronary arteries (pericoronary perivascular adipocytes) exhibit a reduced state of adipogenic differentiation and a heightened proinflammatory state, secreting ≤50-fold higher levels of the proinflammatory cytokine monocyte chemoattractant peptide-1 compared with adipocytes from other regional depots. Thus, perivascular adipocytes may contribute to upregulated inflammation of PVAT observed in atherosclerotic human blood vessels. However, perivascular adipocytes also secrete anti-inflammatory molecules such as adiponectin, and elimination of PVAT in rodent models has been shown to augment vascular disease, suggesting that some amount of PVAT is required to maintain vascular homeostasis. Evidence in animal models and humans suggests that inflammation of PVAT may be modulated by environmental factors, such as high-fat diet and tobacco smoke, which are relevant to atherosclerosis. These findings suggest that the inflammatory phenotype of PVAT is diverse depending on species, anatomic location, and environmental factors and that these differences are fundamentally important in determining a pathogenic versus protective role of PVAT in vascular disease. Additional research into the mechanisms that regulate the inflammatory balance of perivascular adipocytes may yield new insight into, and treatment strategies for, cardiovascular disease.

Key Words: adipocytes ■ adipokines ■ adiponectin ■ chemokine CCL2 ■ inflammation

The traditional model of the pathogenesis of atherosclerosis has focused on intimal disease following the paradigm of endothelial injury, leading to inflammation, monocyte recruitment, and foam cell formation. This view focuses on the luminal aspect of the vessels with the inflammation initiating inside the vessel wall and radiating outward (inside-out model), with adventitia and perivascular adipose tissue (PVAT) occupying a relatively passive role. However, this paradigm has come under intense scrutiny because the extent of adventitial inflammation in atherosclerosis has been better appreciated. In apolipoprotein E–deficient mice, accumulation of T cells and B cells in the lamina adventitia far exceeds that in the intima (≤80-fold higher), and intense clustering of macrophages and lymphocytes was demonstrated at the border between the lamina adventitia and PVAT in atherosclerotic human aorta, suggesting that PVAT could play an active role in promoting vascular inflammation. This view is further supported by observations that balloon or wire injury rapidly induces inflammation and perturbs adipokine gene expression profiles in PVAT in pigs and mice. These and other studies have led to a gradual shift toward an outside-in model of inflammation in vascular disease and placed PVAT prominently in the spotlight of vascular biology.

Adipocytes and Inflammation

Adipocytes are traditionally viewed as energy storage cells that play a key role in energy balance, thermogenesis, and glucose homeostasis. However, adipocytes also produce several soluble factors (adipokines) that regulate inflammation, and they...
possess machinery including toll-like receptors that facilitate inflammatory interactions. Adipokines such as resistin and leptin exert predominantly proinflammatory effects, whereas adiponectin is classically anti-inflammatory. Thus, adipocytes can also be viewed as integral components of the immune system. Evolutionarily, adipose tissue was derived from the primitive fat body of insects, which simultaneously serves both the animal’s metabolic and immune functions. Although higher animals have evolved separate systems to serve these functions (ie, liver and adipose tissues for metabolism and hematopoietic system for immunity), adipose tissue has retained its basic immune functions. The contribution of adipocytes to inflammation of adipose tissues in the pathogenesis of obesity-related disease is well established. Adipose inflammation, in turn, serves as a major driver of systemic inflammation that links obesity to cardiovascular disease. Although proinflammatory adipocytokines are expressed by PV AT, the notion that PV AT may locally contribute to cardiovascular disease is a more recent and less well-founded hypothesis.

Features of PVAT

PVAT is a conglomerate of various cell types, including adipocytes, preadipocytes, and mesenchymal stem cells, embedded in a matrix that is invested with microvessels and which may contain a similar system of sympathetic innervation seen in richly innervated brown adipose tissue (BAT) and white adipose tissue, although evidence on PVAT innervation is scarce. In humans, PVAT directly abuts the adventitia of most large conduit arteries in the body, with the notable exception of the cerebral vasculature, and some microvascular beds such as the mesentery. The absence of a separating fascia layer promotes paracrine communications between PVAT and the associated vasculature (Figure 1). As such, adipocytes from PVAT can infiltrate into the adventitia to facilitate their effects on local inflammation and vascular tone. PVAT has been shown to modulate vascular contractility via paracrine release of various factors including adiponectin. Furthermore, PVAT is anatomically colocalized with atherosclerotic lesions in humans, correlating with plaque burden and vascular calcifications. Similar to visceral and subcutaneous adipose tissues, PVAT expands during obesity with hypertrophy of both brown and white perivascular adipocytes noted in rodent models of high-fat feeding.

Mounting evidence suggests that the phenotype and function of PVAT vary depending on species and anatomic location, displaying features of traditional white adipose tissue versus energy-combusting BAT or an intermediate between the two. Most of the published data pertaining to brown adipose phenotype of PVAT were derived from rodent studies; the extent to which this reflects the phenotype of human PVAT is less clear. Human perivascular adipocytes surrounding coronary arteries exhibit a histological appearance and gene expression pattern more consistent with white rather than brown adipocytes (Figure 1). However, compared with subcutaneous and perirenal adipocytes derived from the same subjects, these perivascular adipocytes are smaller in size, less efficient in storing lipid, and express lower levels of adipocyte-specific genes, suggesting a reduced state of adipogenic differentiation. Global gene expression analyses indicate that these features of perivascular adipocytes map to early developmental differences, consistent with their origination from a distinct precursor cell, as has been proposed for adipose-derived stem cells surrounding perivascular regions of subcutaneous and visceral adipose tissues of mice. In contrast, in vitro differentiated perivascular adipocytes derived from human radial arteries are larger in size and exhibit a high capacity to form lipid droplets, suggesting that the origin and differentiation state of human perivascular adipocytes is variable depending on the vascular bed.

Developmental Origins of Adipocytes

The majority of research in the origin of adipocytes comes from work on subcutaneous and visceral adipocytes isolated from the stromal vascular fraction of homogenized adipose tissues. The emerging consensus is that a majority of the mature adipocytes within each adipose tissue depot derive from precursor cells with distinct embryological lineages. Lineage analysis studies suggest that the source of adipocyte progenitor cells in visceral white adipose tissue depots is heterogeneous, with the lateral plate mesoderm serving as a major contributor and Wilm’s tumor gene 1–positive progenitors giving rise predominately to epididymal as opposed to retroperitoneal fat. In contrast, Wilm’s tumor gene 1 is not expressed in subcutaneous adipose tissue or BAT; BAT seems to originate mainly in the paraxial mesoderm. Adipose stromal cells isolated from subcutaneous adipose tissues of mice were also suggested to be heterogeneous, with a subpopulation shown to have a neural crest origin, termed neural crest–derived adipose stromal cells. These cells were demonstrated to have high adipogenic differentiation potential.
but markedly attenuated osteogenic and chondrogenic potentials compared with their non-neural crest adipose stromal cell counterparts. Studies involving perivascular adipocytes are relatively scarce and, to the best of our knowledge, point toward an origin distinct from other adipocytes. Inferential evidence suggests that these cells may arise from vascular smooth muscle cell progenitors. Furthermore, in a murine model with vascular smooth muscle cell–specific PPAR-γ (peroxisome proliferator activated receptor gamma) deletion, the animals had normal white adipose tissue and BAT depots but lacked PVAT. Although far from definitive proof, the above data suggest a common embryological origin for perivascular adipocytes and vascular smooth muscle cell.

**Nature Versus Nurture: Adipocyte Plasticity**

Multiple studies support the idea that the surrounding microenvironment is a critical factor in cell lineage determination of adipose stromal cells. In addition, mature adipocytes have been suggested to possess the ability to dedifferentiate into lipid-free fibroblast-like cells, which, under proper induction culture in vivo or in vitro, demonstrated adipogenic, osteogenic, and chondrogenic potentials similar to adipose stromal cells isolated from stromal vascular fraction. Thus, the microenvironment may also regulate the differentiation state and phenotype of mature adipocytes. The function of brown adipocytes is generally linked to thermogenesis and temperature homeostasis, whereas white adipocytes are viewed to be important in energy storage and systemic endocrine functioning. However, this dichotomy has recently been challenged with the discovery of brite (brown-in-white) adipocytes detected in white adipose depots, which may contribute to temperature homeostasis and whole-body metabolism in humans. A bidirectional interconversion between brite adipocytes and brown adipocytes was demonstrated in inguinal fat depots in mice. Cold exposure has been shown to stimulate brown adipocyte hyperplasia and increase metabolic activity in rodent models. In humans, short-term cold exposure similarly increased 2-[18F]fluoro-2-deoxyglucose uptake in BAT; positron emission tomography images raised the possibility of 2-[18F]fluoro-2-deoxyglucose uptake into fat surrounding the heart and great vessels. Whether cold exposure directly or indirectly affects the function or phenotype of perivascular adipocytes remains to be determined.

Other dietary cues may also regulate the function of perivascular adipocytes. Chatterjee et al showed that feeding mice a high-fat diet for just 2 weeks produced marked reduction in the expression of adipocyte-related genes (including the anti-inflammatory adipokine adiponectin) in thoracic PVAT, suggesting a reduction in the state of adipogenic differentiation. Furthermore, expression of the proinflammatory cytokine macrophage inflammatory protein-1α was markedly upregulated in the PVAT, whereas expression in subcutaneous and visceral depots was minimally affected. These changes occurred before significant recruitment of inflammatory cells to the PVAT depot, suggesting that the perivascular adipocytes themselves responded adversely to the high-fat diet. Similarly, high-fat diet disrupted the balance between oxidant production and antioxidant defense mechanisms in murine PVAT; the oxidant stress resulting from this imbalance could contribute to amplification of inflammation in the PVAT depot. Consistent with this notion, mice deficient in adipose triglyceride lipase, a key lipolytic enzyme that regulates lipid accumulation in adipose tissues, exhibited marked expansion of the thoracic aorta PVAT depot along with amplification of oxidative stress and inflammation. Interestingly, perivascular adipocytes (perimesenteric) from smokers, who also exhibit increased oxidative stress, expressed higher levels of monocytic chemotactic peptide-1 (MCP-1) mRNA and enhanced activity of the P2X,R (purinergic receptor P2X ligand channel 7 receptor)–inflammome complex compared with adipocytes isolated from nonsmokers. Taken together, these data suggest that adipocytes exhibit considerable plasticity in response to environmental cues, and responsiveness of perivascular adipocytes to noxious stimuli contained in high-fat diet and tobacco smoke may promote a proinflammatory state conducive to the development of atherosclerosis.

**Regulation of Inflammation by Perivascular Adipocytes**

The inflammatory state of human PVAT has been the subject of intense research in the past decade. Many studies have shown a heightened state of inflammation in PVAT surrounding atherosclerotic blood vessels, both in terms of inflammatory cell infiltration and proinflammatory gene expression. However, PVAT is composed of many cell types, so the specific contribution of perivascular adipocytes to the global inflammatory state of the tissue is impossible to infer from these studies. Only a few publications have investigated the inflammatory potential of perivascular adipocytes, either freshly isolated or in vitro differentiated. Therefore, the information presented here contains the most current knowledge on this topic and is inherently limited in scope. Nevertheless, these data suggest that human perivascular adipocytes surrounding conduit arteries possess intrinsic proinflammatory characteristics. Our laboratory demonstrated that human pericoronary perivascular adipocytes expressed high levels of MCP-1, interleukin-8, and interleukin-6, which play a critical role in inflammatory cell recruitment to adipose tissues and are also important in the pathobiology of vascular disease. In particular, release of MCP-1 protein from in vitro differentiated perivascular adipocytes was 50-fold greater than that from adipocytes derived from other regional depots of the same subjects. Human periradial adipocytes similarly expressed higher levels of proinflammatory MCP-1 and proangiogenic mediators compared with visceral or subcutaneous adipocytes. In a wire-induced injury model in mice, Jabs et al showed sequential expression of MCP-1 and other cytokines/cytokine receptors in perivascular tissue starting on the adventitial aspect of the vessel and progressing toward the intima. MCP-1 gene deletion in animal models led to substantial reductions in arterial wall macrophage infiltration and lipid deposition. Conditioned media from perivascular adipocytes elicited chemotaxis of granulocytes, monocytes, and activated T cells and stimulated vascular smooth muscle cell proliferation, which supports a pathological role for cytokines and growth factors produced by perivascular adipocytes.

Conversely, pericoronary perivascular adipocytes secreted markedly less adiponectin (an anti-inflammatory adipokine)
compared with their subcutaneous and perirenal counterparts, which is further consistent with a proinflammatory phenotype. It is important to point out that the PVAT was obtained from previously healthy organ donors without a pre-existing history of heart disease, suggesting that the proinflammatory phenotype was a fundamental property of the cells and not the product of an atherosclerotic milieu. Extending these studies, we performed genome-wide analyses comparing human subcutaneous and perivascular adipocytes and showed that perivascular adipocytes had a strikingly higher expression of genes involved in proinflammatory pathways, including cell adhesion molecules and complement system factors such as complement 7, complement H, SERPINE2 (serine peptidase inhibitor), SERPINE1, and cytosolic PLA2 (phospholipase A2). The latter study, the most differentially upregulated gene expressed by perivascular adipocytes was tumor necrosis factor receptor superfamily member 11b (osteoprotegerin). Osteoprotegerin is a secreted protein that is best known as a decoy ligand for RANK (receptor activator of NFKB) that regulates osteogenesis, but it also exerts both proinflammatory and anti-inflammatory effects, and its levels are elevated in humans with atherosclerosis and mice fed a high-fat diet. Compared with subcutaneous adipocytes, perivascular adipocytes secreted markedly higher levels of osteoprotegerin protein, raising the possibility that PVAT contributes importantly to systemic osteoprotegerin levels in obesity and atherosclerosis. The functional importance of proinflammatory mediator secretion by perivascular adipocytes was demonstrated using a transendothelial monocyte migration assay. Compared with conditioned medium from subcutaneous adipocytes, medium from perivascular adipocytes induced a 2-fold increase in monocytic cell migration across an endothelial monolayer, supporting a proinflammatory phenotype of human pericoronary perivascular adipocytes.

However, our microarray data also showed increased expression of certain anti-inflammatory genes by the perivascular adipocytes, including tumor necrosis factor α-induced protein 6 or tumor necrosis factor α–stimulated protein 6 and suppressor of cytokine signaling 2, which suggests that perivascular adipocytes can also exert anti-inflammatory effects. A protective effect of PVAT is similarly inferred by the observation that removal of PVAT from the femoral artery enhanced neointima formation after wire injury in high-fat–fed mice, suggesting that the presence of some amount of PVAT is protective and needed to maintain vascular homoeostasis. Interestingly, in the latter study, replacement of PVAT with subcutaneous fat from mice fed a chow diet reduced neointima formation, whereas transplanted subcutaneous fat from high-fat–fed mice was without effect, which further suggests a link between high-fat diet and regulation of vascular disease by PVAT. In contrast to these findings, transplanting visceral fat to the murine carotid artery (which is normally devoid of PVAT) enhanced atherosclerosis, mediated in part via P-selectin glycoprotein ligand-1. It must be pointed out that none of the aforementioned studies transplanted authentic PVAT, which differs phenotypically from subcutaneous and other visceral fat depots, so the results of these studies may not be directly applicable to endogenous PVAT. With regard to the effects of dietary fat on PVAT, mice fed a long-term high-fat diet demonstrated reduced macrophage infiltration into regions of brown PVAT, and atherosclerosis was enhanced in mice devoid of PVAT that were housed under conditions of low temperature, most likely because of loss of the systemic metabolic effects of brown PVAT during adaptive thermogenesis. These studies suggest that the inflammatory phenotype of PVAT is diverse depending on species, anatomic location, and environmental conditions and that these differences are fundamentally important in determining a pathogenic versus protective role of PVAT in vascular disease.

**Perspective: Balancing Proinflammatory Versus Anti-Inflammatory Effects of PVAT**

The available data, when interpreted in total, suggest that PVAT is capable of both positively and negatively regulating vascular inflammation to modulate vascular disease (Figure 2). Data in human perivascular adipocytes suggest that MCP-1 is likely to be an important proinflammatory mediator that is expressed at high levels and may contribute to vascular disease. Perivascular adipocytes also secrete proinflammatory adipokines and express complement system factors and cell adhesion/matrix proteins that may facilitate inflammation. Several anti-inflammatory molecules likely counterbalance the proinflammatory state of human perivascular adipocytes. Data in rodent models clearly indicate that PVAT secretes anti-inflammatory factors that can favorably influence vascular pathophysiology. Several questions remain unanswered, such as how perivascular adipocytes interact with inflammatory cells and what role the nervous system might play in regulating PVAT inflammation. Additional research into the mechanisms that regulate the inflammatory balance of perivascular adipocytes may yield new insight into, and treatment strategies for, cardiovascular disease.

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**Disclosures**

None.
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