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The Role of Chemokines in Recruitment of Immune Cells to the Artery Wall and Adipose Tissue

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Abstract

The role of the immune system is to recognize pathogens, tumor cells or dead cells and to react with a very specific and localized response. By taking advantage of a highly sophisticated system of chemokines and chemokine receptors, leukocytes such as neutrophils, macrophages, and T-lymphocytes are targeted to the precise location of inflammation. While this is a beneficial process for acute infection and inflammation, recruitment of immune cells to sites of chronic inflammation can be detrimental. It is becoming clear that these inflammatory cells play a significant role in the initiation and progression of metabolic disorders such as atherosclerosis and insulin resistance by infiltrating the artery wall and adipose tissue (AT), respectively. Data from human studies indicate that elevated plasma levels of chemokines are correlated with these metabolic diseases. Recruitment of macrophages to the artery wall is well known to be one of the first steps in early atherosclerotic lesion formation. Likewise, recruitment of macrophages to AT is thought to contribute to insulin resistance associated with obesity. Based on this knowledge, much recent work in these areas has focused on the role of chemokines in attracting immune cells (monocytes/macrophages in particular) to these 2 sites. Thus, understanding the potential for chemokines to contribute to metabolic disease can help direct studies of chemokines as therapeutic targets. In this article, we will review current literature regarding the role of chemokines in atherosclerosis and obesity-related insulin resistance. We will focus on novel work showing that chemokine secretion from endothelial cells, platelets, and adipocytes can contribute to immune cell recruitment, with a diagram showing the time course of chemokine expression and leukocyte recruitment to AT. We will also highlight a few of the less-commonly known chemokine-chemokine receptor pairs. Finally, we will discuss the potential for chemokines as therapeutic targets for treatment of atherosclerosis and insulin resistance.

Keywords

macrophages; leukocytes; cardiovascular disease; atherosclerosis; insulin resistance

I. INTRODUCTION

Classical chemokines are small 8–10 kDa proteins that act as chemotactic molecules for various cell types. There are about 50 known chemokines divided into different families based upon

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the separation of the first 2 cysteine residues. Other molecules such as lipids (leukotrienes, prostaglandins, and lysophospholipids), complement factors (C3a and C5a), and small peptides (defensins) can also act as chemoattractants. The majority of chemokine receptors are G-protein coupled receptors, and with about 20 known chemokine receptors and extensive overlap of ligand-receptor recognition, the chemokine system has the capacity to be very specific, yet very complex. The chemokine-chemokine receptor system functions in many physiological processes such as development, wound repair, and immunity. Increasingly, it is becoming understood that chemokines can also play a role in pathological processes such as autoimmunity, tumorigenesis, atherosclerosis, and insulin resistance. Overreaction by the immune system to chronic inflammatory stimulation can result in inappropriate leukocyte recruitment to the artery wall, resulting in atherosclerotic lesion formation, or to AT, leading to insulin resistance. The purpose of this article is to describe the current literature on the role of classical chemokines in leukocyte recruitment to the artery wall and to AT, with a focus on targeting chemokines as potential therapeutic targets for these metabolic diseases.

II. CHEMOKINES IN THE ARTERY WALL ASSOCIATED WITH ATHEROSCLEROSIS

Analysis of human and mouse atheroma samples has revealed the presence of many different chemokines that are selectively expressed in diseased areas (Tedgui and Mallat, 2006). In addition, with the development of atherosclerotic-prone mouse models such as the low density lipoprotein receptor deficient (LDLR^{-/-}) and apolipoprotein E deficient (apoE^{-/-}) mice, many different studies have yielded critical information regarding the role of chemokines in atherosclerotic lesion formation as will be described below.

A. CC Chemokines and their Receptors

CC chemokines belong to the β -chemokine family where the first two N-terminal cysteine residues are adjacent to one another. Monocyte chemoattractant proteins (MCP), macrophage inflammatory proteins (MIP), and regulated upon activation, normal T cell expressed and secreted (RANTES) are all CC chemokines. These chemokines attract most monocytes, eosinophils, basophils, and lymphocytes. They can also attract neutrophils under certain conditions.

i. MCP-1 and CCR2—MCP-1 and its receptor CCR2 have been repeatedly implicated in atherosclerotic lesion development. Multiple studies have demonstrated that when expression of MCP-1 (CCL2 in standard nomenclature) or its receptor CCR2, is removed, mice are relatively protected against lesion formation. Two groups have demonstrated that mice deficient in both CCR2 and apoE (CCR2^{-/-};apoE^{-/-} mice) have smaller aortic root lesions than apoE^{-/-} control mice despite an absence of change in plasma lipids (Boring et al., 1998; Dawson et al., 1999). Deletion of MCP-1 also decreases atherosclerotic plaque burden, as shown in mice that overexpress human apoB (Gosling et al., 1999). Conversely, using bone marrow transplantation to overexpress MCP-1 on hematopoietic cells increases atherosclerotic lesion area in apoE^{-/-} mice (Aiello et al., 1999).

Because removal of MCP-1 and CCR2 consistently reduces atherosclerotic lesions in various mouse models, this pathway is considered a potential target for pharmacological intervention in atherosclerosis. An N-terminal deletion mutant of MCP-1 has been used as effective gene therapy in apoE^{-/-} mice and is able to limit the destabilization of preexisting atherosclerotic plaques even though it does not reduce lesion size (Inoue et al., 2002). However, targeting a single chemokine pathway—even a prominent pathway such as MCP-1/CCR2—in humans may not have the desired protective effects. Blockade of CCR2 using the selective CCR2

receptor antagonist INCB-3344 induces elevated plasma MCP-1 and, unexpectedly, does not alter atherosclerosis in apoE^{-/-} mice (Aiello et al., 2009).

ii. MIP-1 α and MIP-1 β and their receptors CCR1 and CCR5—In addition to MCP-1, MIP-1 α (CCL3) and MIP-1 β (CCL4) with their receptors CCR1 and CCR5 are also involved in atherosclerotic lesion formation. CCR5 deficiency reduces atherosclerotic lesion area and inflammation in both apoE^{-/-} and LDLR^{-/-} mice (Potteaux et al., 2006; Braunersreuther et al., 2007; Quinones et al., 2007), although CCR5 deficiency is not protective against early atherosclerosis in apoE^{-/-} mice (Kuziel et al., 2003). Furthermore, CCR5 deficiency protects against arterial wire injury induced neointima formation in apoE^{-/-} mice (Zernecke et al., 2006). Some of these beneficial effects can be accounted for by the upregulation of anti-inflammatory cytokine IL-10 in CCR5^{-/-} mice (Zernecke et al., 2006; Braunersreuther et al., 2007). The atherogenic effects of CCR5 appear to be independent of MCP-1 and the chemokine receptor CX₃CR1, because CCR5 antagonism further decreases lesion area in apoE^{-/-};MCP-1^{-/-};CX₃CR1^{-/-} mice (Combadiere et al., 2008). In contrast to CCR5, studies of atherosclerosis prone mice have shown a detrimental effect of CCR1 deficiency on lesion area (Potteaux et al., 2005; Braunersreuther et al., 2007), suggesting that CCR1 may be protective against lesion formation.

iii. RANTES and its receptors—RANTES (CCL5) is expressed by endothelial cells, smooth muscle cells, platelets, and macrophages. It can bind to and signal through CCR1, CCR3, and CCR5. RANTES functions primarily by aggregating and binding to glycosaminoglycans on the endothelial cell surface, resulting in a localized, potent chemotactic signal. For this reason, RANTES has long been considered as a potential target to reduce macrophage infiltration into the artery wall. Dominant negative peptides of RANTES have been used to determine the effects of RANTES inhibition on atherosclerosis. MetRANTES is a recombinantly produced RANTES receptor antagonist that has been shown to reduce atherosclerotic lesion formation in LDLR^{-/-} mice without altering plasma lipid levels (Veillard et al., 2004). These effects appear to be due to displacement of several different chemokine ligands of CCR1 and CCR5 resulting in reduced macrophage and T-lymphocyte content within the lesions. Another form of RANTES, [⁴⁴AANA⁴⁷]-RANTES, is deficient in its ability to bind to glycosaminoglycans as well as to aggregate but can still act as a chemoattractant. [⁴⁴AANA⁴⁷]-RANTES reduces atherosclerosis in LDLR^{-/-} mice, presumably by oligomerizing with RANTES and preventing binding and aggregation of RANTES to glycosaminoglycans on the endothelial cell surface (Braunersreuther et al., 2008). [⁴⁴AANA⁴⁷]-RANTES has also recently been shown to be cardioprotective during early myocardial reperfusion by decreasing expression of MCP-1 and MIP-1 α , as well as by decreasing overall levels of oxidative stress and apoptosis in the reperfused myocardium (Braunersreuther et al., 2009).

iv. CCL19 and CCL21 and their receptor CCR7—CCR7 is observed in human atherosclerotic plaques, especially in T-lymphocyte rich areas, and CCL19 (MIP-3 β) and CCL21 (SLC) are present in both the macrophages and T-lymphocytes within human plaques (Damas et al., 2007). CCR7 and its ligands are important for the ability of T-lymphocytes to emigrate from peripheral tissues (Debes et al., 2005), and they may similarly enable emigration from the artery wall. When atherosclerotic arterial segments from apoE^{-/-} mice are grafted into wild type mice, they abundantly express CCR7, resulting in decreased lesion size and foam cell content. This decrease in lesion area is greatly blunted when CCR7 signaling is interrupted by the presence of CCL19 and CCL21 antibodies (Trogan et al., 2006). Thus, CCR7 may have an atheroprotective role, and care should be taken to avoid inhibition of CCR7 when targeting CC receptors as a therapy for atherosclerosis.

v. Inhibition of CC chemokines for treatment of atherosclerosis—A few specific antagonists of specific chemokines and their receptors have been used for the treatment of experimental atherosclerosis with some level of success, as described above. Beyond these studies, use of the broad-spectrum CC chemokine inhibitor, NR58-3.14.3 results in reduced macrophage content and cleaved collagen in apoE^{-/-} mice (Reckless et al., 2005). For future studies, combined targeting of multiple chemokine/chemokine receptor pathways or targeting of other atherosclerosis risk factors with supplementation of chemokine inhibition may be effective alternatives.

B. CXC chemokines and their receptors

CXC chemokines belong to the α -chemokine family and are characterized by a separation of the first two N-terminal cysteine residues with a non-cysteine amino acid. Chemokines such as stromal derived factor-1 (SDF-1), monocyte induced by interferon γ (MIG), IL-8 and the growth-regulated oncogene (GRO) family members are all CXC chemokines. These chemokines preferentially chemoattract neutrophils and lymphocytes depending on the sequence preceding the CXC sequence, and are therefore involved early in the inflammatory sequence of events.

i. CXCL9, CXCL10, and CXCL11 and their receptor CXCR3—T helper lymphocytes, which participate in the pro-inflammatory changes within the artery wall that initiate atherosclerotic plaque development, characteristically express CXCR3, the receptor for CXCL9 (Mig), CXCL10 (IP-10), and CXCL11 (I-TAC). All three of these CXCR3 ligands are highly expressed in human atherosclerotic lesions (Mach et al., 1999). Heparin, although frequently used for its anticoagulant capacity, is also anti-inflammatory and can decrease T-lymphocyte trafficking into lesions, likely by competing with CXCR3 for the binding of its ligands (Ranjbaran et al., 2006). ApoE^{-/-};CXCR3^{-/-} mice have less lipid deposition in their thoracoabdominal aortas than apoE^{-/-} controls, coupled with an increase in anti-inflammatory molecules and a greater number of regulatory T-lymphocytes (Veillard et al., 2005). This suggests that the presence of CXCR3 may favor the recruitment of regulatory T-lymphocytes, which, because of their immunosuppressive role, may be protective in the early stages of atherosclerosis (Veillard et al., 2005). In a recent study, King and colleagues have shown that CXCL10 is upregulated in Ang-II induced atherosclerosis in apoE^{-/-} mice (King et al., 2009). Furthermore, they showed that deficiency of CXCL10 decreased atherosclerotic plaque size but increased aortic aneurysm formation in apoE^{-/-} mice. Thus, CXCL10 may play different roles in vascular disease, both promoting atherosclerotic lesion formation and protecting against aneurysm formation. With regards to therapies targeting CXCR3, the specific antagonist, NBI-74330, has been shown attenuate atherosclerotic lesion formation in LDLR^{-/-} mice (van Wanrooij et al., 2008).

ii. CXCL16 and its receptor CXCR6—CXCL16 (SR-PSOX) has dual properties in that it can act both as a scavenger receptor for oxidized low density lipoproteins and as an inflammatory chemokine, making it of special interest to atherosclerosis research. It is a transmembrane protein that can be cleaved by ADAM10 to yield soluble CXCL16 (sol-CXCL16), which can activate CXCR6⁺ T-lymphocytes. CXCL16 and CXCR6 are known to be increased in atherosclerotic plaques (Minami et al., 2001; Wuttge et al., 2004), although the role of CXCL16 in cardiovascular disease has been debated ((Sheikine et al., 2006) and it's two associated Letters to the Editor). Plasma sol-CXCL16 is elevated in patients with CAD or acute coronary syndrome relative to control patients (Lehrke et al., 2007), and circulating CXCL16 is related to the severity of coronary artery stenosis (Yi and Zeng, 2008). In mouse studies, LDLR^{-/-} mice that are deficient in CXCL16 have increased atherosclerotic lesion area, suggesting that CXCL16 is atheroprotective (Aslanian and Charo, 2006). As more studies

are conducted regarding CXCL16 and cardiovascular disease, its contribution to atherosclerosis will hopefully become clearer.

C. CX₃CL1 and its receptor

The chemokine fractalkine (CX₃CL1), which is in a distinct class from the CC and CXC chemokines, also affects lesion size and progression. This has been demonstrated in a triple knockout mouse model in which fractalkine deficiency further reduces atherosclerotic lesion area and macrophage accumulation in apoE^{-/-} and in LDLR^{-/-} mice (Teupser et al., 2004). A novel antagonist of CX₃CR1, a CX₃CL1 analog called F1, has recently been shown to potently inhibit activation of the CX₃CR1 signaling pathway, and may hold promise for treatment of atherosclerosis (Dorham et al., 2009).

III. SOURCES OF CHEMOKINES IN ATHEROSCLEROTIC DISEASE

Many different cell types contribute to the formation of atherosclerotic lesions. While lipid-laden macrophages within the intima are the hallmark of early lesions, they would not acquire access to the artery wall if it were not for endothelial cells and platelets. Furthermore, the AT surrounding both the vessel wall and the heart may contribute to the pathogenesis of atherosclerotic disease. While there are many mechanisms by which endothelial cells, platelets and adipocytes can influence disease progression, this review will focus on their ability to secrete chemokines; thus leading to recruitment of leukocytes to the artery wall.

A. Endothelial cell derived chemokines

Leukocyte recruitment into tissues involves a multi-step process: rolling along the endothelial cell layer, activation, adhesion, and transendothelial migration (reviewed in (Langer and Chavakis, 2009)). Chemokines are involved in this process in several ways. First, chemokines secreted from other cell types can adhere to glycosaminoglycans on the surface of endothelial cells within the vessel lumen. This allows for a stationary and localized formation of chemoattractant gradients, drawing leukocytes to sites of inflammation where they can form firm adhesions before transmigrating into the underlying tissue. Second, chemokines can be secreted by endothelial cells. The secretion of chemokines by endothelial cells has been shown to be promoted by several different stimuli relevant to cardiovascular disease. Inflammatory stimuli such as bacterial lipopolysaccharide as well as cytokines such as interleukin-1 β and tumor necrosis factor- α , have been shown to induce MCP-1 expression in human umbilical vein endothelial cells (HUVECs) (Rollins et al., 1990; Sica et al., 1990; Liu et al., 2009). In addition to inflammatory molecules, growth factors have also been shown to induce chemokine secretion from endothelial cells. For example, vascular endothelial cell growth factor strongly induces the expression and secretion of interleukin-8 (IL-8/CXCL8) in human umbilical artery endothelial cells (HUAECs) and HUVECs (Hao et al., 2009).

Conditions specific to human metabolic disorders can also potentiate the activation of endothelial cells leading to chemokine secretion. For example, advanced glycation end products are elevated in individuals with diabetes, and incubation of HUVECs with these advanced glycation end products induces expression of IL-8 and MCP-1 (Liu et al., 2009). Angiotensin II (Ang-II), known for its ability to increase blood pressure, has been shown to induce chemotaxis of neutrophils and mononuclear cells. By signaling through the Ang-II type I receptor, Ang-II upregulates the synthesis of IL-8 in HUAECs. CXCR2, the receptor for IL-8, is necessary for subsequent synthesis and secretion of the CC chemokines MCP-1, MIP-1 α , and RANTES (Abu Nabah et al., 2007). As the main leukocytes that attach to arterial endothelium are mononuclear, and both mononuclear cells and HUAECs express CXCR2, this data indicates that Ang-II could induce secretion of CC chemokines by either mononuclear cells or endothelial cells. Ang-II also upregulates secretion of CXCL10 by human endothelial

cells in vitro, and this data has been verified in vivo by showing that mice infused with Ang-II have increased expression of CXCL10 (Ide N et al 2008). Therefore, Ang-II may contribute to the accumulation of monocytes/macrophages in the artery wall by inducing release of IL-8 and CXCL10 by endothelial cells. Statins also influence the inflammatory nature of endothelial cells. Human vascular endothelial cells have reduced expression of MCP-1, MIP-1 β , and CCR4 when treated with 1 μ M simvastatin via inhibition of the geranylgeranylpyrophosphate pathway (Veillard NR et al 2006). In addition, ortho-hydroxy atorvastatin, an atorvastatin metabolite, significantly decreases secretion of growth-related oncogen α (GRO α /CXCL1) from HUVECs without lowering GRO α mRNA expression (Breland et al., 2008). Finally, Hastings *et al.* have shown that human endothelial cells cultured in “atheroprone” conditions of low shear stress and disturbed flow patterns, have phenotypic changes resulting in increased mRNA expression and protein secretion of the chemokine IL-8 (Hastings et al., 2007). The authors speculate that endothelial cell secretion of IL-8 could induce a migratory phenotype in nearby smooth muscle cells. Because MCP-1 is a potent chemoattractant for monocytes and IL-8 is a strong chemoattractant for neutrophils and lymphocytes, the secretion of these chemokines by endothelial cells could mediate recruitment of many different leukocytes to the artery wall during atherosclerotic lesion formation.

A very intriguing new field of investigation is the chemokine-mediated recruitment of adult endothelial progenitor cells and smooth muscle progenitor cells to the artery wall during atherosclerotic plaque, neointima, and collateral formation. This process has been recently reviewed (Hristov and Weber, 2009), and thus will not be covered in the current article. While these endothelial progenitor cells are thought to have a heart-protective function by promoting neovascularization and collateral formation, they have also been shown to be atherogenic (George et al., 2005), possibly because they secrete significant amounts of chemokines such as MCP-1, IL-8, and RANTES (Zhang et al., 2009).

B. Platelet derived chemokines

Platelets store many different chemokines including MIP-1 α , RANTES, CCL7, CCL17, CXCL1, CXCL4, CXCL5, and IL-8 in their α -granules for release upon activation. Platelets can be activated by thrombin, oxLDL, and CD40 ligand to release these chemokines, which are then retained on the vascular endothelial cell layer. Upon activation of platelets, platelet-derived microparticles are also formed. These platelet-derived microparticles can actually transfer chemokine receptors such as CXCR4 as well as adhesion molecules to leukocytes, resulting in the adherence of the leukocytes to the endothelial cell layer (Janowska-Wieczorek et al., 2001). Two of the predominant chemokines found in platelets, RANTES and CXCL4, have been shown to form heterodimers, which increase the RANTES-mediated arrest of monocytes on endothelial cells (von Hundelshausen et al., 2005). In fact, disruption of RANTES-CXCL4 heterodimers by means of a specific peptide (comprised of residues 25–44 of RANTES in a stable, cyclic form) reduces atherosclerotic lesion area in apoE $^{-/-}$ mice without compromising general functioning of the immune system (Koenen et al., 2009). In addition, platelets can activate endothelial cells to secrete MCP-1 and IL-8 by signaling through CD40 ligand on the activated platelet surface (Henn et al., 1998). Thus, both by releasing chemokines that then become bound to endothelial cells and by activating endothelial cells to secrete other chemokines, platelets have a potent role in the recruitment of leukocytes to the artery wall. In fact, when platelet adhesion to vascular endothelium does not occur, atherosclerotic lesion formation is almost completely ameliorated in apoE $^{-/-}$ mice (Massberg et al., 2002; Huo et al., 2003).

C. Perivascular, epicardial, and visceral AT derived chemokines

Another potential contributor to atherosclerosis is AT, both the AT near atherosclerotic lesions, such as perivascular AT (pAT) and epicardial AT (eAT), as well as visceral AT (vAT). pAT

lies adjacent to the adventitia of the artery wall, and in mice, the amount of pAT increases with high fat diet feeding (Henrichot et al., 2005). As its name suggests, eAT surrounds the heart, and its thickness is significantly greater in obese versus normal weight humans (Iacobellis et al., 2007). Importantly, in humans both pAT and eAT are sources of chemokines and contribute to inflammation in atherogenic regions.

A recent comparison of adipocytes in pAT compared to those in subcutaneous AT (sAT) and perirenal AT demonstrated some distinguishing properties of the adipocytes adjacent to the artery wall (Chatterjee et al., 2009). Adipocytes in human pAT are smaller and more irregularly shaped than subcutaneous or perirenal adipocytes. In vitro differentiated perivascular adipocytes have lower expression of adipogenic regulatory genes and decreased secretion of adiponectin and leptin, indicating that they are not as mature/differentiated as other adipocytes. However, perivascular adipocytes do secrete MCP-1 and IL-8, and may release more of these chemokines than adipocytes from other depots (Chatterjee et al., 2009). Furthermore, there appears to be a difference in the adipocyte phenotype in pAT coming from the abdominal aorta compared with the thoracic aorta. Abdominal pAT from obese mice contains elevated levels of MCP-1 and more macrophages in comparison to abdominal pAT from lean mice, and these differences are not detected in pAT from the thoracic area (Police et al., 2009). pAT surrounding atherosclerotic regions of the aorta contains dramatically more CD68+ macrophages and CD3+ T-lymphocytes than pAT covering normal arteries (Henrichot et al., 2005), and differential secretion of chemokines likely contributes to this.

Human eAT has greater mRNA expression of inflammatory genes including numerous chemokines (MCP-1, MIP-1 α , MIP-1 β , RANTES, CCL11, CCL18, CCL21, CXCL1, CXCL2) and chemokine receptors (CCR2, CXCR4, CXCR6) as compared to sAT (Mazurek et al., 2003). Release of RANTES from human eAT, but not from other depots, positively correlates with the metabolic risk factors of body mass index, plasma triglycerides, and diastolic blood pressure (Madani et al., 2009). This data suggests that eAT, like pAT, may be involved in the chemokine-chemokine receptor inflammatory signaling occurring during cardiovascular disease. In summary, eAT thickness and inflammatory nature have been associated with obesity-related insulin resistance and cardiovascular disease (Iacobellis and Leonetti, 2005; Iacobellis et al., 2007), but its role in initiating or propagating these diseases remains unknown.

In addition to pAT and eAT, vAT is also highly inflammatory and may play a relevant role in atherosclerotic lesion formation. ApoE $^{-/-}$ mice transplanted with vAT have larger atherosclerotic lesions than sham-operated mice, but in contrast, transplantation of sAT does not increase atherosclerosis. A significant elevation in plasma MCP-1 is present in the mice transplanted with vAT, and as this is the only noted difference between the groups, it likely contributes to the observed increase in atherosclerosis (Ohman et al., 2009). Thus, various fat depots contribute distinctly to cardiovascular health, and chemokines secreted from the AT mediate some of these properties.

IV. CHEMOKINES IN HUMAN ATHEROSCLEROTIC DISEASE

While chemokine expression from various cells in the artery wall contribute to leukocyte recruitment and promote atherosclerotic disease, plasma levels of various chemokines have also been shown to correlate with human cardiovascular diseases. Furthermore, evidence shows that statin treatment to reduce plasma lipids has pleiotropic effects by influencing chemokine levels.

A. Predictive and diagnostic potential of chemokine levels for cardiovascular disease

Several chemokines are significantly elevated in the circulation of individuals with coronary artery disease, and although increased inflammation is not unique to atherosclerosis, a recent

publication suggests measurement of a combination of chemokines can be helpful for predicting disease. Inclusion of circulating CXCL10, MIP-1 α , and CCL7 with the traditional risk factors of waist circumference and smoking in a linear regression model has better diagnostic potential than linear regression models based only on traditional risk factors (Ardigo et al., 2007). In patients with unstable angina pectoris, circulating MIP-1 α , RANTES, and CCL18 transiently increase during ischemia, and plasma MIP-1 α is a potential predictor of future ischemic events (Kraaijeveld et al., 2007; de Jager et al., 2008). Plasma MIP-1 α as well as MCP-1 and RANTES are also elevated in patients with severe acute myocardial infarction (Parissis et al., 2002; Kobusiak-Prokopowicz et al., 2007). These and other clinical studies demonstrate that measurement of circulating chemokines may assist in early detection of cardiovascular disease and improve patient care.

Secretion of chemokines by peripheral blood mononuclear cells (PBMCs) is altered by some cardiovascular diseases. PBMCs from coronary artery disease patients spontaneously secrete more MCP-1, IL-8, GRO α , CXCL9, and CXCL10 than PBMCs from controls, and they release greater amounts of MCP-1 and IL-8 when stimulated with oxLDL (Breland et al., 2008; de Oliveira et al., 2009). Unstimulated PBMCs from familial hypercholesterolemia patients release more MIP-1 α , MIP-1 β , and IL-8 than control PBMCs, and this spontaneous release is positively correlated with plasma total and LDL cholesterol (Holven et al., 2003). It is possible that MIP-1 α , MIP-1 β , and IL-8 are especially important mediators of inflammation in individuals with familial hypercholesterolemia and, more generally, in people with high LDL cholesterol. An altered pattern of chemokine secretion by PBMCs in individuals with cardiovascular disease may contribute to the differences in both circulating and local chemokine concentrations that are characteristic of this malady.

B. Statins as a therapeutic option for chemokine-targeted therapy

Although statins are primarily used to lower circulating LDL cholesterol levels, they have independent effects on inflammation. Pitavastatin (2 mg daily) given for 6 months to hyperlipidemic patients not only improved plasma cholesterol but also increased plasma adiponectin. When separate data analysis was performed on those whose plasma adiponectin levels increased from baseline by at least 1.5 times, a significant decrease in plasma MCP-1 was noted (Nomura et al., 2009). Thus, it appears that pitavastatin can induce an adiponectin-dependent effect on systemic MCP-1 in addition to its lipid lowering capacity. Atorvastatin (80 mg daily) significantly downregulates the CXC chemokines GRO α , GRO β , and GRO γ in PBMCs drawn from coronary artery disease patients after 6 months of statin treatment (Breland et al., 2008). Although both plasma LDL and expression of GRO α in PBMCs from atorvastatin treated patients are decreased with statin therapy, they are not correlated, indicating that atorvastatin's effect on GRO α may be distinct from its effects on lipid metabolism. In vitro studies using human monocytes also demonstrate a reduction in the secretion of CC and CXC chemokines by statins (Bruegel et al., 2006; Breland et al., 2008; Montecucco et al., 2009).

V. CHEMOKINES IN ADIPOSE TISSUE ASSOCIATED WITH INSULIN RESISTANCE

The past decade has been marked by a revolution in the field of AT biology because of the discovery that macrophages accumulate in AT in obesity (Weisberg et al., 2003; Xu, H et al., 2003). Not only does obese AT contain more macrophages, the expression of chemokines and their receptors are also upregulated in AT from obese mice (Xu, H et al., 2003) and humans (Dahlman et al., 2005; Huber et al., 2008). Furthermore, these AT macrophages (ATMs) appear to secrete the majority of inflammatory cytokines that are derived from AT and their accumulation precedes the development of systemic insulin resistance. Because of the

pathophysiological relevance of these ATMs, much work has focused on determining the mediators of macrophage recruitment to AT.

A. MCP-1 and its receptor CCR2

To date, MCP-1 and its receptor CCR2 are the best-studied of the chemokine ligands and receptors for their role in macrophage accumulation in AT and obesity-induced insulin resistance. MCP-1 is upregulated at the mRNA and protein levels in mesenteric, epididymal, subcutaneous, and perirenal AT of diet induced obese mice (Yu et al., 2006). F4/80 and CD68 expression is also increased in these AT depots (Yu et al., 2006). In addition, *in vitro* migration experiments show increased macrophage migration to mesenteric AT-conditioned media, and an antibody for MCP-1 blocks this migration (Yu et al., 2006).

Two different groups have engineered transgenic mice using the human adipocyte fatty acid binding protein (aP2) gene promoter to overexpress MCP-1 in AT (Kamei et al., 2006; Kanda et al., 2006). AT of the MCP-1 overexpressing mice contains more macrophages than AT from wild type mice, and MCP-1 transgenic mice are insulin resistant and glucose intolerant, suggesting that MCP-1 may promote macrophage infiltration and insulin resistance during obesity. In addition, attenuation of MCP-1 activity with a dominant negative approach, improved insulin sensitivity and glucose tolerance in obese leptin receptor deficient and diet induced obese mice (Kanda et al., 2006). On the other hand, experiments with MCP-1^{-/-} mice have yielded inconsistent results. Kanda et al. found that diet induced obesity in MCP-1^{-/-} mice resulted in fewer ATMs and improved insulin sensitivity and glucose tolerance, without a difference in body weight relative to wild type controls (Kanda et al., 2006). In contrast, Inouye et al. observed significantly increased body weight in diet induced obese MCP-1^{-/-} mice and possibly a slight increase in macrophage accumulation in AT combined with decreased insulin sensitivity (Inouye et al., 2007). Kirk et al. found similar results to Inouye et al. except that they observed no differences in insulin or glucose tolerance (Kirk et al., 2008). CCR2 deficient mice have been shown to have attenuated diet induced obesity and reduced ATM accumulation by two groups (Weisberg et al., 2006; Lumeng et al., 2008) but to have no effect on these parameters by a third group (Inouye et al., 2007). However, pharmacological inhibition of CCR2 with INCB3344 (Weisberg et al., 2006) or propagermanium (Ito et al., 2008; Tamura et al., 2008) reduces macrophage infiltration of adipose tissue in obese mice. While care should be taken when comparing genetic knockout models for chemokine deficiency with pharmacological antagonists, these studies indicate that reduction of MCP-1 or CCR2 alone may not be enough to alleviate macrophage recruitment to AT, and that more broad-spectrum inhibitors of several different chemokine/chemokine receptors will be necessary.

B. Other chemokines in AT

Research on the role of chemokines in macrophage recruitment to AT is so new that many of these chemokines have been studied and published by only one group to date. In the models described below, mice have been placed on high fat diets to determine the role of various chemokines on ATM accumulation in obesity.

i. CCL20 and its receptor CCR6—In a study to determine the role of chemokines in the recruitment of T-lymphocytes to AT, Duffaut *et al.* (Duffaut et al., 2009b) showed that CCL20 is released from adipocytes and induces the migration of CD4⁺ T-lymphocytes that express its receptor, CCR6. Expression of CCL20 from adipocytes correlates with body mass index in humans. These data provide evidence that chemokines may be involved not only in recruiting macrophages but also T-lymphocytes to AT.

ii. CXCL14—CXCL14 is a chemokine for macrophages and dendritic cells; however, its receptor has not yet been identified. CXCL14 was originally cloned and studied as a molecule involved in tumorigenesis, but it has been discovered that CXCL14 is expressed in AT, and its expression level is increased in obese mice (Nara et al., 2007). Furthermore, CXCL14^{-/-} mice have a reduced number of macrophages in their AT, which corresponds with reduced hepatic steatosis and improved muscle insulin sensitivity.

iii. CXCL5 and keratinocyte-derived chemokine and their receptor CXCR2—

There have been 2 very recent reports on the potential role of CXCL5 and keratinocyte-derived chemokine (KC) and their receptor CXCR2 in mediating ATM accumulation. Chavey *et al.* (Chavey et al., 2009) showed that CXCL5 (traditionally regarded as a chemoattractant for neutrophils) is highly expressed in AT compared to liver and muscle and that its expression is derived from ATMs. In obese subjects, plasma levels of CXCL5 are elevated. Blocking CXCL5 with antibodies or an antagonist, as well as removal of CXCR2 by gene targeting in mice, results in improved insulin sensitivity. Like CXCL14, CXCL5 appears to promote insulin resistance by interfering with insulin signaling pathways in muscle. Thus, CXCL5 and CXCL14 may provide a direct link whereby an ATM-derived chemokine impacts systemic insulin sensitivity.

KC is the mouse orthologue of human IL-8 and is a chemoattractant for neutrophils and monocytes and can induce adhesion of monocytes to the endothelial cell layer, promoting atherogenesis. Neels and colleagues have studied KC in AT and found that its expression is increased in obesity (Neels et al., 2009). Furthermore, they show that bone marrow transplantation of CXCR2 deficient marrow (to induce an absence of leukocyte responses to KC) results in reduced numbers of ATMs and improved insulin sensitivity. However, CXCR2 is a receptor for many different chemokines (Table 1); thus, whether the results found in the CXCR2^{-/-} bone marrow transplant model are mediated by absence of a response to KC or some other CXC chemokine is unknown.

C. Predictive and diagnostic potential of chemokine levels for insulin resistance

As mentioned above, plasma levels of chemokines have been shown to be predictive of cardiovascular disease. Recent studies have also shown that elevated plasma levels of chemokines such as MCP-1 and IL-8 are associated with obesity and its related metabolic abnormalities such as inflammatory cytokines and reduced high density lipoprotein levels (Kim et al., 2006). Furthermore, sAT expression of the chemokine receptors, CCR1, CCR3, and CCR5 are highly correlated with plasma free fatty acid and fasting insulin levels (Huber et al., 2008).

VI. Sequence of events in leukocyte recruitment to the artery wall and AT

Many groups have contributed to our current understanding of the role of chemokines in leukocyte recruitment to the artery wall and AT. This process in atherosclerosis development has been well described by Libby and colleagues (Weber et al., 2008), therefore we will focus on the sequence of events that occurs in AT. Figure 1 illustrates the sequence in which chemokines are expressed and leukocytes infiltrate AT during high fat diet feeding.

An elevation in chemokines is one of the first events observed in AT of mice fed a high fat diet, and many chemokines are upregulated in the AT during the progression of obesity. An increase in MCP-1 expression in AT has been shown to occur as early as 1 day following high fat diet feeding, and this is soon followed by expression of MCP-2 and MCP-3. This early increase in MCP expression is concomitant with an influx of neutrophils into AT. Expression of chemokines during weeks 1–12 following high fat diet feeding has not been extensively studied; however it is during this time that cytotoxic CD8⁺ T-cells and macrophages begin to

accrue in the AT (Nishimura et al., 2009). Furthermore, the presence of helper CD4+ T-cells begins to decline during this time (Nishimura et al., 2009). The expression of other chemokines such as MIP-1 α , MIP-1 β , and RANTES is elevated at a later time point (12–16 weeks) and continues to be elevated through 26 weeks of high fat diet feeding (Xu, H. et al., 2003; Jiao et al., 2009; Nishimura et al., 2009). Until a more thorough time course analysis is performed, it is difficult to speculate which chemokines are predominantly responsible for recruitment of different leukocyte populations to the AT.

VII. Similarities and Differences of Chemokine role in Artery Wall and AT

Leukocyte infiltration of the artery wall has frequently served as a model of what might occur in AT during the onset of obesity. However, important differences between these two tissues are apparent. For example, within AT, leukocytes circulate through small blood vessels and capillaries before extravasating into the tissue. In contrast, atherosclerosis occurs primarily in the aorta (in mice) and in coronary arteries (in humans) in which blood is circulating with more force and in greater volume. In addition, during obesity, AT is actively growing to accommodate more lipid storage whereas the increased lipid storage in atherosclerosis is ectopic and pathogenic. Furthermore, the populations of adipocytes proximal to the artery wall versus adipocytes in omental or subcutaneous AT, for example, have distinct inflammatory properties (Chatterjee et al., 2009). All of these differences could account for distinct roles of chemokines in each disease. It should be noted that there are many differences in potential mediators of macrophage recruitment to AT versus recruitment to the artery wall. For example, fatty acid flux, adipokines, hypoxia, and adipocyte cell death are all thought to play a role in initiating macrophage recruitment to AT but may not be relevant to atherogenesis. In contrast, the presence of hyperlipidemia or oxLDL may significantly impact atherosclerosis, but they don't seem to affect ATM accrual (Coenen et al., 2007). As research in this field continues, it will be important to distinguish between the contributions of specific chemokines to the inflammation in the artery wall versus in AT.

Due to the limited information on different chemokine/chemokine receptors in AT, MCP-1 is the only chemokine with which enough information is available to begin to make comparisons of the role of chemokines in these two tissues. Despite the fact that investigators initially hypothesized that there were many similarities in mechanisms leading to recruitment of macrophages to artery wall and AT, the literature suggests that the role of MCP-1 differs in these two tissues. In AT, MCP-1 is upregulated at an early time point in high fat diet fed mice, and studies have shown that removal of MCP-1 and CCR2 can reduce AT inflammation and insulin resistance (Kanda et al., 2006). However, conflicting reports have been published and AT inflammation and insulin resistance also occur independently of MCP-1/CCR2 (Inouye et al., 2007; Kirk et al., 2008; Lumeng et al., 2008). Because MCP-1 is expressed so early after high fat diet feeding, it might not be an effective target in individuals who are already obese. A more consistent picture emerges when considering MCP-1 as a target for atherosclerosis reduction. Blockade of MCP-1 can interfere with the progression of preexisting atherosclerosis (Inoue 2002), suggesting that MCP-1 may one day become part of an effective intervention to delay or stop the progression of atherosclerosis, however, data is lacking that MCP-1 inhibition could result in lesion regression.

VII. CONCLUSIONS

Chemokines play important roles in many different physiologic and pathophysiologic processes. In this review, we have tried to cover the most recent discoveries on the role of chemokines in atherosclerosis and in insulin resistance, based upon their ability to recruit leukocytes to the artery wall and AT, respectively. Current research has identified plasma chemokines as a likely tool for improved cardiovascular disease diagnosis models and

prediction of future ischemic events. More research will be required to determine whether inhibition of chemokines will be a reasonable therapeutic tool for treating metabolic diseases.

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ABBREVIATIONS USED

apoE ^{-/-}	apolipoprotein E deficient mouse
AT	adipose tissue
ATM	adipose tissue macrophage
AngII	angiotensin II
eAT	epicardial adipose tissue
HUAEC	human umbilical artery endothelial cells
HUVEC	human umbilical vein endothelial cells
IL	interleukin
LDLR ^{-/-}	low density lipoprotein receptor deficient mouse
oxLDL	oxidized LDL
pAT	perivascular adipose tissue
PBMCs	peripheral blood mononuclear cells
sAT	subcutaneous adipose tissue
vAT	visceral adipose tissue

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Figure 1. Regulation of specific chemokines and leukocytes in AT of high fat diet fed mice

The diagram indicates only the initial upregulation of each chemokine during high fat diet feeding and is based on the following references: (Jiao et al., 2009; Nishimura et al., 2009). Numbers on blue arrows represent the number of weeks on high fat diet. The initial reported increase or decrease in each cell type is shown; the horizontal arrows indicate that high fat diet fed mice continue to have more macrophages and CD8+ T-lymphocytes and fewer CD4+ T-lymphocytes and Treg lymphocytes throughout high fat diet feeding. Additional sources used: (Weisberg et al., 2003; Xu, H. et al., 2003; Caspar-Bauguil et al., 2005; Coenen et al., 2007; Strissel et al., 2007; Takahashi et al., 2007; Rausch et al., 2008; Duffaut et al., 2009a; Feuerer et al., 2009; Winer et al., 2009)

Note to editor: Please combine these two slides into on figure with this panel below the first slide.

Table 1

Nomenclature for chemokines mentioned in this article

CC Name	Common Name	Other Names	Receptors	Present in lesions	Present in AT	Special notes
CCL2	MCP-1		CCR2, CCR4	yes	yes	
CCL3	MIP-1 α	LDL78	CCR1, CCR3, CCR5	yes	yes	stored in platelet α -granules
CCL4	MIP-1 β	HL-MAP	CCR5	yes	yes	
CCL5	RANTES		CCR1, CCR3, CCR5	yes	yes	stored in platelet α -granules, MetRANTES and [⁴⁴ AANA ⁴⁷]-RANTES are inhibitors
CCL7	MCP-3		CCR1, CCR2, CCR3	yes	yes	stored in platelet α -granules
CCL11	eotaxin		CCR3	yes	yes	
CCL17		TARC	CCR4	yes	NR	stored in platelet α -granules
CCL18		PARC	unknown	yes	yes	
CCL19	MIP-3 β	ELC	CCR7	yes	yes	
CCL20	MIP-3 α	LARC	CCR6	ND	yes	
CCL21		SLC	CCR7	yes	yes	
CXCL1	GRO α		CXCR2/IL8RB	yes	yes	stored in platelet α -granules, KC is the mouse homologue
CXCL2	GRO β		CXCR2/IL8RB	yes	yes	MIP-2 is the mouse homologue
CXCL3	GRO γ	MIP-2b	CXCR2/IL8RB	NR	NR	expressed in HUVECs
CXCL4	PF4		CXCR3-B	yes	NR	stored in platelet α -granules
CXCL5		ENA-78	CXCR2/IL8RB	NR	yes	stored in platelet α -granules
CXCL8	IL-8		CXCR1/IL8RA, CXCR2/IL8RB	yes	yes	only in humans, stored in platelet α -granules
CXCL9	Mig		CXCR3	yes	NR	
CXCL10	IP-10		CXCR3	yes	yes	
CXCL11	I-TAC		CXCR3	yes	NR	

CC Name	Common Name	Other Names	Receptors	Present in lesions	Present in AT	Special notes
CXCL14	MIP-2 γ	BRAK, BMAK	unknown	NR	yes	
CXCL16		SR-PSOX	CXCR6	yes	NR	
CX ₃ CL1	fractalkine		CX ₃ CR1	yes	NR	

NR: not reported
ND: not detected