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Monocyte-Endothelial Cell Interactions in the Development of Atherosclerosis

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Abstract

The activation of endothelial cells at atherosclerotic lesion-prone sites within the vessel results in the upregulation of cell adhesion molecules and chemokines, which mediate the recruitment of circulating monocytes. Accumulation of monocytes and monocyte-derived phagocytes in the wall of large vessels leads to chronic inflammation and the development and progression of atherosclerosis. This review discusses the nature of these molecules and the mechanisms involved in the early steps of monocyte recruitment into atherosclerotic lesion sites within the vessel wall.

Introduction

Cardiovascular disease is the leading cause of death of both men and women in the United States. Nearly three-fourths of all deaths from cardiovascular disease are due to heart attack or stroke caused by atherosclerosis, a chronic inflammatory disease of the arterial wall, characterized by the formation of lipid-laden lesions. Although the initiation of atherosclerotic disease is strongly correlated with prolonged hyperlipidemia, it has become increasingly evident that there is an immunological component to the development and progression of the disease, as suggested by the accumulation of leukocytes in atherosclerotic lesions(Galkina et al. 2007a). In fact, recruitment of monocytes to the vessel wall is an early step in the formation of atherosclerotic lesions, the importance of which is supported by many studies(Gerrity 1981, Ross 1993). The molecular mechanisms involved in the recruitment of monocytes by activated endothelial cells at sites of atherosclerotic lesion formation are similar to those reported for neutrophils and lymphocytes (Galkina et al. 2007b), but some molecules are of specific importance in monocyte recruitment.

Adhesion Cascade

The recruitment of circulating monocytes occurs via a tightly regulated multi-step process mediated by a combination of cell surface adhesion molecules. Initially, activated endothelial cells at sites of incipient atherosclerosis express P-selectin, which mediates the tethering and rolling of circulating monocytes. P-selectin binds to P-selectin glycoprotein ligand-1 (PSGL-1) and other glycosylated ligands on monocytes(Elstad et al. 1995, Weyrich et al. 1995). PSGL-1, the dominant ligand for all three selectins, is only functional when properly glycosylated. PSGL-1 binds P-selectin in the presence of fucosyl transferase, sialyl transferase, core2 GlcNAc transferase and sulfatyl transferase activities(McEver et al. 1995). All these enzymes are constitutively expressed in monocytes(Ley et al. 2004), yielding constitutively active

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PSGL-1. There is some evidence that E-selectin is also inducibly expressed at sites of atherosclerosis(van der Wal et al. 1992), and E-selectin deficiency in ApoE-null mice had a modest effect on lesion development compared to ICAM-1 and P-selectin deficiency(Collins et al. 2000). However, functional data in relevant models that would directly support a role for this molecule are lacking.

Under normal blood flow, selectin-mediated interactions are not sufficient to arrest rolling leukocytes. It is now evident that selectins not only allow the capturing and rolling of leukocytes on the endothelium, but they also signal through PSGL-1 to activate integrins and induce monocyte activation(Weyrich et al. 1995, Ma et al. 2004). Integrins are heterodimeric cell surface receptors and support both rolling and adhesion of leukocytes. Upon activation, integrins undergo a series of conformational changes that result in increased binding affinity for their respective ligands(Luo et al. 2007). The monocyte integrin most relevant in atherosclerosis is VLA-4, also known as α4β1 integrin(Huo et al. 2001a). α4β1 mediates rolling on its ligand vascular cell adhesion molecule-1 (VCAM-1) when in a lower affinity conformation(Alon et al. 1995) and firm adhesion when in the high affinity state. VCAM-1 is a member of the immunoglobulin-like superfamily of adhesion molecules and, although not routinely expressed under physiological conditions, is induced on cytokine-stimulated endothelium. Monocytes, like lymphocytes, express VLA-4 and the β2 integrin LFA-1 constitutively, and like neutrophils, express L-selectin, as well as P- and E-selectin ligands (Imhof et al. 2004). Several lines of evidence suggest that VLA-4 is a major ligand mediating rolling and firm adhesion of monocytes to inflamed endothelium. Much of the functional work on the monocyte recruitment mechanisms has been completed in mice lacking the cholesterol acceptor protein apolipoprotein E (*Apoe*$^{-/-}$) on chow or high-fat diet(Zhang et al. 1992), or in low density lipoprotein receptor knockout mice (*Ldlr*$^{-/-}$) on high-fat diet(Ishibashi et al. 1993). *Ex vivo* studies using carotid arteries isolated from atherosclerotic-prone *Apoe*-deficient mice on high fat diet showed that the rolling velocities of perfused mononuclear cell lines increased and monocyte adhesion was reduced by 75% after monoclonal antibody blockade of VLA-4 or VCAM-1(Ramos et al. 1999, Huo et al. 2000). In a similar study, Huo et al. showed that firm arrest of monocytes on early atherosclerotic endothelium was mainly mediated by chemokine-triggered activation of VLA-4(Huo et al. 2001b). In addition, Gerszten et al. showed that endothelial cells transfected with VCAM-1 supported monocyte rolling and firm adhesion, while reconstituted *in vitro* systems using monocytes on cytokine-activated endothelial cells under shear flow suggested the involvement of P-selectin, L-selectin, VCAM-1, and VLA-4(Luscinskas et al. 1994, Gerszten et al. 1998).

Integrin activation is typically mediated by signals induced by chemokine receptor engagement that triggers arrest and firm adhesion(Campbell et al. 1998, Weber 2003, Smith et al. 2004, Smith et al. 2005). Some chemokines can bind to the surface of endothelial cells, immobilize and mediate arrest of rolling leukocytes. These arrest chemokines bind heparan sulfate, a glycosaminoglycan present on the surface of endothelial cells. The association of chemokines with heparan sulfate immobilizes chemokines on the vessel wall, providing strong and localized signals for integrin activation(Weber et al. 1999). The GRO family of CXC chemokines, CXCL1, -2 and -3(Huo et al. 2001b, Smith et al. 2005), interleukin-8 (CXCL8) (Gerszten et al. 1999), CCL5(Huo et al. 2003), alone or as a heterodimer with CXCL4(von Hundelshausen et al. 2005), have all been shown to arrest monocytes on activated endothelium. In addition, certain chemokines can bind Duffy antigen receptor for chemokines (DARC), a molecule expressed on red blood cells and endothelial cells, which binds and presents chemokines at the endothelial cell surface(Pruenster et al. 2006). Leukocytes rolling along the endothelium bind these immobilized chemokines and are arrested owing to full activation of their integrin molecules.

Monocyte Recruitment

LDL Accumulation and Endothelial Cell Activation

Atherosclerotic lesions are thought to start by subendothelial low-density lipoprotein (LDL) accumulation, which leads to endothelial cell (EC) activation and chronic inflammation. The endothelium is the primary barrier between blood and tissues. However, ECs are susceptible and sensitive to the shear stresses provided by blood flow, which can change their morphology and trigger many signaling cascades(World et al. 2006, Chiu et al. 2008). LDL accumulation preferentially occurs at sites of arterial branching or curvature, where flow is disturbed, in contrast to areas of continuous laminar flow, which are not or less affected. There is a positive correlation between areas of low shear stress and sites of LDL accumulation and lesion initiation(Zand et al. 1999). Hyperlipidemia, due to both genetic and environmental factors increases the risk of atherosclerosis, as greater circulating LDL levels lead to increased accumulation.

Oxidation is one of the many modifications that LDL can undergo once trapped in the vessel wall. Stimulation of arterial EC by accumulated oxidized LDL (oxLDL) induces endothelial cell activation and the expression of many pro-inflammatory genes. Among these, the adhesion molecules P-selectin, VCAM-1 and ICAM-1 have proven to be important in atherosclerotic lesion development(Dong et al. 1998, Shih et al. 1999, Collins et al. 2000). P-selectin has been shown to be induced on EC by stimulation with oxidized, but not native forms of LDL *in vitro* (Gebuhrer et al. 1995). These and other studies have failed to show similar results for E-selectin expression(Gebuhrer et al. 1995, Khan et al. 1995). Studies in mice deficient in both P- and E-selectin suggest overlapping functions of P- and E-selectins in the development of atherosclerosis(Dong et al. 1998), but data interpretation is confounded by the generally poor health of these mice(Bullard et al. 1996, Subramaniam et al. 1996, Dong et al. 1998).

Adhesion Molecules

VCAM-1 is required for the slow rolling of monocytes and plays an important role in the initial steps of monocyte recruitment to atherosclerotic lesions. The expression of VCAM-1 and intercellular adhesion molecule-1 (ICAM-1), a ligand for β2 integrins, are augmented on EC by oxLDL upon TNFα induction *in vitro*(Khan et al. 1995), and both molecules have been shown to be present on EC in atherosclerotic plaques *in vivo*(Cybulsky et al. 1991, Poston et al. 1992). The functional importance of VCAM-1 expression in atherosclerotic lesions is supported by studies in which monocyte adhesion to endothelial cells in carotid arteries from atherosclerotic-prone *Apoe*-deficient mice was significantly inhibited by monoclonal antibody blockade of VLA-4 or VCAM-1(Huo et al. 2000). Furthermore, activation of VLA-4 by CXCL1, a pro-inflammatory chemokine found in atherosclerotic lesions, mediated the firm adhesion of monocytes on EC(Huo et al. 2001b). Mice lacking ICAM-1 expression had significantly smaller lesions than wild-type mice on high-fat diets(Nageh et al. 1997), but the exact role of ICAM-1 in monocyte recruitment has not been defined. Unlike VCAM-1, the expression on ICAM-1 is not correlated to lesion sites(Cybulsky et al. 2001). Since ICAM-1 and its β2 integrin ligands are not involved in monocyte arrest from flow(Huo et al. 2001b), ICAM-1 is believed to participate in adhesion strengthening, monocyte spreading and transendothelial migration. This hypothesis is supported by a number of *in vitro* studies (Luscinskas et al. 1994).

Chemokines and Cytokines

Activated endothelial cells express a number of chemokines that affect the recruitment of monocytes to the vessel wall. Among them, CCL2 and CCL5 have been shown to participate in the development of atherosclerotic lesions. *In vivo* studies using mice genetically deficient in CCL2 or its cognate receptor, CCR2, in atherosclerosis-prone strains have demonstrated

significant protection against lesion formation, accompanied by a decrease in subendothelial monocyte accumulation(Boring et al. 1998, Gu et al. 1998, Combadiere et al. 2008). Compared to *Ccl2*$^{-/-}$ mice, *Ccr2*$^{-/-}$ mice have little to no circulating Ly-6C^{high} monocytes(Jia et al. 2008). These data suggest that CCR2 expression on monocytes mediates the mobilization of monocytes from the bone marrow during an inflammatory response, and that other CCR2 ligands can compensate for the lack of CCL2. Decreased monocyte accumulation in *Ccr2*$^{-/-}$ mice may therefore be indirectly due to fewer circulating monocytes. CCL5 is deposited by activated platelets onto inflamed endothelium in *Apoe*$^{-/-}$ mice on high fat diets(von Hundelshausen et al. 2001, Huo et al. 2003). Mice deficient in the CCL5 receptor, CCR5, on *Apoe*$^{-/-}$ background have reduced lesion formation and lesional macrophage content (Braunersreuther et al. 2007). Furthermore, treatment with a CCL5 antagonist also inhibits leukocyte recruitment into atherosclerotic plaques(Braunersreuther et al. 2008). Although a number of chemokines have been shown to arrest rolling monocytes at the endothelial cell surface(Gerszten et al. 1999, von Hundelshausen et al. 2001, von Hundelshausen et al. 2005), they may also transmit chemotactic signals that direct monocytes into the vessel wall. In contrast, the growth-related oncogene(Lortat-Jacob et al. 2002) family of CXCR2 ligands probably act solely as arrest chemokines, mediating the firm adhesion of rolling monocytes on VCAM-1 under flow in the presence of P-selectin(Smith et al. 2005). Interestingly, a non-canonical CXCR2 ligand and pro-inflammatory cytokine, migration inhibitory factor (MIF), acts as an arrest chemokine and plays a critical role in the progression of atherosclerotic lesions (Bernhagen et al. 2007).

It has become evident that monocytes are a heterogeneous population of cells. The blood monocyte population consists of at least two, and possibly more, subsets(Sunderkotter et al. 2004). In mice, the expression levels of the chemokine receptor CX3CR1 and the GPI-anchored surface molecules Ly-6C and Ly-6G, recognized by mAb Gr-1, distinguish these subsets. One type is characterized by a more inflammatory phenotype and is CX3CR1^{low} Gr-1^{high}, whereas the other type is believed to be a precursor for tissue-resident macrophages and dendritic cells, and is CX3CR1^{high} Gr-1^{low}. CX3CR1 expression also defines two major monocyte subsets in humans, CD14^{+}CD16^{-} and CD14^{lo}CD16^{+}(Geissmann et al. 2003). In an imaging-based study using isolated blood monocytes, inflammatory monocytes are preferentially recruited to atherosclerotic lesions(Swirski et al. 2007). They express higher levels of PSGL-1 than do Gr-1^{low} monocytes and exhibit enhanced binding to P- and E-selectin. In an *ex vivo* perfused carotid artery model, Gr-1^{high} monocytes interacted preferentially with atherosclerotic endothelium compared with Gr-1^{low} monocytes in a PSGL-1–dependent manner(An et al. 2008). Several studies have provided evidence to support the idea that the Gr-1^{high} inflammatory monocytes are immature cells that leave the bone marrow and mature in the circulation, converting to the Gr-1^{low} phenotype(Sunderkotter et al. 2004, Tacke et al. 2007). Gr-1 downregulation may be part of the monocyte to macrophage/dendritic cell differentiation process; however, it is not clear whether differentiation occurs before or after entering atherosclerotic lesions.

The chemokine receptor, CX3CR1, plays a major role in the development of atherosclerosis. Studies in independently derived CX3CR1-deficient mice on *Apoe*$^{-/-}$ backgrounds have shown significant protection against atherosclerotic lesion development(Combadiere et al. 2003, Lesnik et al. 2003). Curiously, *Cx3cr1*$^{+/-}$ *Apoe*$^{-/-}$ mice were protected similarly to the *Cx3cr1*$^{-/-}$ *Apoe*$^{-/-}$ mice. However, the mechanism by which CX3CR1 promotes atherosclerosis progression is not fully understood. Although CX3CR1 binds to the tethered chemokine CX3CL1, and endothelial cells at sites of atherosclerosis express some mCX3CL1, most of this chemokine is expressed in the smooth muscle cell layer. There is no *in vivo* evidence that would support a direct role of CX3CR1 in monocyte arrest and adhesion, although this is a widely assumed model based on *in vitro* data(Fong et al. 1998, Haskell et al. 1999, Schulz et al. 2007). CX3CL1-mediated adhesion requires its aggregation at the cell surface

(Hermand et al. 2008). Deletion of CX3CL1 dramatically decreased macrophage accumulation and development of atherosclerosis in *Ccr2*$^{-/-}$ mice; however, circulating monocytes were not reduced below the already low level in *Ccr2*$^{-/-}$ mice, suggesting a role in direct recruitment (Saederup et al. 2008). Interestingly, CX3CR1 is also expressed on activated ECs, and may play a role in strengthening of adhesive interactions between monocytes and activated ECs. ICAM-1 upregulation on EC by CX3CR1 stimulation has been seen in human coronary artery and umbilical vein endothelial cells, and may be one of many genes regulated by CX3CR1 (Yang et al. 2007).

Mice with null mutations in monocyte colony stimulating factor M-CSF (*Csf1*$^{-/-}$)(Smith et al. 1995) or its receptor (*Csf1r*$^{-/-}$) also show dramatically reduced atherosclerotic lesion sizes in various models. Interestingly, even the heterozygous M-CSF-deficient mice exhibit reduced atherosclerotic lesions(Rajavashisth et al. 1998). The role of M-CSF and M-CSFR is thought to be related to monocyte maturation, differentiation and macrophage survival in the vessel wall, rather than directly participating in recruitment. In fact, *Csf1*$^{-/-}$ mice have severely impaired production of blood monocytes, and deficiency of peritoneal and tissue macrophages. These mice require feeding of a special liquid diet due to lack of osteoclasts, which results in osteopetrosis and an inability of teeth to erupt(Qiao et al. 1997).

Platelet Activation

As briefly mentioned, platelet activation promotes the adhesive interaction between monocytes and endothelial cells during atherosclerosis. It is believed that aggregation of activated platelets with circulating monocytes promotes monocyte-endothelial interactions by deposition of pro-inflammatory chemokines and P-selectin expression(Huo et al. 2004). Indeed, CCL5 deposition by platelets on activated endothelium induces arrest of rolling monocytes(von Hundelshausen et al. 2001, Huo et al. 2003). In addition, the combination of platelet-derived platelet factor 4 (PF4/CXCL4) with CCL5 results in an effect greater than CCL5 alone(von Hundelshausen et al. 2005). Furthermore, P-selectin-mediated rolling of activated platelets on inflamed endothelium is important for the progression of atherosclerotic lesions. Although some ECs express functional PSGL-1(Rivera-Nieves et al. 2006), the endothelial ligand for platelet P-selectin in atherosclerotic lesions is unknown. P-selectin-expressing platelets injected into *Apoe*$^{-/-}$ mice accelerated the formation of atherosclerotic lesions(Huo et al. 2003), whereas injection of P-selectin-deficient platelets resulted in smaller lesions. This finding is supported by bone marrow transplantation experiments suggesting that platelet and not endothelial P-selectin drives atherosclerosis(Burger et al. 2003).

Conclusion

Atherosclerosis is a complex disease in which the immune cells play a critical role. The recruitment of monocytes is an important step in the development of atherosclerotic plaques. OxLDL-mediated activation of endothelial cells at lesion-prone sites in the vasculature initiates an inflammatory response, which leads to the expression of P-selectin, VCAM-1 and chemokines necessary for the recruitment of monocytes into the vessel wall. Understanding the regulation of these molecules will help us to determine the keys to the specific homing of monocytes to the arterial wall and may provide insights that could lead to the development of therapies to combat atherosclerotic lesion formation.

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