apoptosis signaling in endothelial progenitor cells. FASEB J 19:974–976.

Walter DH, Haendeler J, Reinhold J, et al: 2005. Impaired CXCR4 signaling contributes to the reduced neovascularization capacity of endothelial progenitor cells from patients with coronary artery disease. Circ Res 97:1142–1151.

Winnier GE, Kume T, Deng K, et al: 1999. Roles for the winged helix transcription factors MF1 and MFH1 in cardiovascular development revealed by nonallelic noncomplementation of null alleles. Dev Biol 213:418–431.

Xue Y, Cao R, Nilsson D, et al: 2008. FOXC2 controls Ang-2 expression and modulates angiogenesis, vascular patterning, remodeling, and functions in adipose tissue. Proc Natl Acad Sci U S A 105:10167–10172.

PII S1050-1738(08)00126-6 TCM

Monocyte-Endothelial Cell Interactions in the Development of Atherosclerosis

Javier Mestas, Klaus Ley*

The activation of endothelial cells at atherosclerotic lesion–prone sites in the arterial tree results in the up-regulation 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 arteries 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. (Trends Cardiovasc Med 2008;18:228–232) © *2008, Elsevier Inc.*

• 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 myocardial infarction 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 immunologic 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 (ECs) 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.

Javier Mestas and Klaus Ley are at the La Jolla Institute for Allergy and Immunology, La Jolla, CA 92037, USA

* Address correspondence to: Dr. Klaus Ley, La Jolla Institute for Allergy and Immunology, 9420 Athena Circle, La Jolla, CA 92037, USA. Tel.: (+1) 858 752 6661; fax: (+1) 858 752 6986.; e-mail: klaus@liai.org.

1050-1738/08/$-see front matter

• Adhesion Cascade

The recruitment of circulating monocytes occurs via a tightly regulated multistep process mediated by a combination of cell surface adhesion molecules. Initially, activated ECs 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). P-selectin glycoprotein ligand-1, the dominant ligand for all 3 selectins, is only functional when properly glycosylated. P-selectin glycoprotein ligand-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 and Kansas 2004), yielding constitutively active 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 intercellular adhesion molecule 1 (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 very late

antigen-4 (VLA-4), also known as $\alpha 4\beta 1$ integrin (Huo and Ley 2001). $\alpha 4\beta 1$ 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. Vascular cell adhesion molecule 1 is a member of the immunoglobulin-like superfamily of adhesion molecules and, although not routinely expressed under physiologic conditions, is induced on cytokine-stimulated endothelium. Monocytes, like lymphocytes, express VLA-4 and the $\beta 2$ integrin LFA-1 constitutively, and like neutrophils, express L-selectin, as well as P- and E-selectin ligands (Imhof and Aurrand-Lions 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 (LDL) receptor knockout mice (*Ldlr*$^{-/-}$) on high-fat diet (Ishibashi et al. 1993). Ex vivo studies using carotid arteries isolated from atherosclerosis-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. (2001) showed that firm arrest of monocytes on early atherosclerotic endothelium was mainly mediated by chemokine-triggered activation of VLA-4. In addition, Gerszten et al. showed that ECs transfected with VCAM-1 supported monocyte rolling and firm adhesion, whereas reconstituted in vitro systems using monocytes on cytokine-activated ECs 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, 2005). Some chemokines can bind to the surface of ECs, immobilize, and mediate arrest of rolling leukocytes. These arrest chemokines bind heparan sulfate, a glycosaminoglycan present on the surface of ECs. 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, CXCL2, and CXCL3 (Huo et al. 2001, 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, a molecule expressed on red blood cells and ECs, which binds and presents chemokines at the EC surface (Pruenster and Rot 2006). Leukocytes rolling along the endothelium bind these immobilized chemokines and are arrested owing to full activation of their integrin molecules.

• Monocyte Recruitment

Low-Density Lipoprotein Accumulation and EC Activation

Atherosclerotic lesions are thought to start by subendothelial LDL accumulation, which leads to 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). Low-density lipoprotein 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 because 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 induces EC activation and the expression of many proinflammatory 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

Vascular cell adhesion molecule 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 ICAM-1, a ligand for $\beta 2$ integrins, are augmented on EC by oxidized LDL 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 and Gimbrone 1991, Poston et al. 1992). The functional importance of VCAM-1 expression in atherosclerotic lesions is supported by studies in which monocyte adhesion to ECs in carotid arteries from atherosclerosis-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 proinflammatory chemokine found in atherosclerotic lesions, mediated the firm adhesion of monocytes on EC (Huo et al. 2001). 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). Because ICAM-1 and its $\beta 2$ integrin ligands are not involved in monocyte arrest from flow (Huo et al. 2001), 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 ECs 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 with *Ccl2*$^{-/-}$ mice, *Ccr2*$^{-/-}$ mice have few 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 EC surface (Gerszten et al. 1999, von Hundelshausen et al. 2001, 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 noncanonical CXCR2 ligand and proinflammatory cytokine, macrophage migration inhibitory factor, 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 2 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 down-regulation 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). *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 ECs at sites of atherosclerosis express some CX3CL1, 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 up-regulation on EC by CX3CR1 stimulation has been seen in human coronary artery and umbilical vein ECs 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 because of 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 ECs during atherosclerosis. It is believed that aggregation of activated platelets with circulating monocytes promotes monocyte-endothelial interactions by deposition of proinflammatory chemokines and P-

selectin expression (Huo and Ley 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 and Wagner 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. Oxidized LDL–mediated activation of ECs 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.

References

Alon R, Kassner PD, Carr MW, et al: 1995. The integrin VLA-4 supports tethering and rolling in flow on VCAM-1. J Cell Biol 128: 1243–1253.

An G, Wang H, Tang R, et al: 2008. P-selectin glycoprotein ligand-1 is highly expressed on Ly-6Chi monocytes and a major determinant for Ly-6Chi monocyte recruitment to sites of atherosclerosis in mice. Circulation 117:3227–3237.

Bernhagen J, Krohn R, Lue H, et al: 2007. MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. Nat Med 13:587–596.

Boring L, Gosling J, Cleary M, Charo IF: 1998. Decreased lesion formation in CCR2−/− mice reveals a role for chemokines in the initiation of atherosclerosis. Nature 394:894–897.

Braunersreuther V, Zernecke A, Arnaud C, et al: 2007. Ccr5 but not Ccr1 deficiency reduces development of diet-induced atherosclerosis in mice. Arterioscler Thromb Vasc Biol 27:373–379.

Braunersreuther V, Steffens S, Arnaud C, et al: 2008. A novel RANTES antagonist prevents progression of established atherosclerotic lesions in mice. Arterioscler Thromb Vasc Biol 28:1090–1096.

Bullard DC, Kunkel EJ, Kubo H, et al: 1996. Infectious susceptibility and severe deficiency of leukocyte rolling and recruitment in E-selectin and P-selectin double mutant mice. J Exp Med 183:2329–2336.

Burger PC, Wagner DD: 2003. Platelet P-selectin facilitates atherosclerotic lesion development. Blood 101:2661–2666.

Campbell JJ, Hedrick J, Zlotnik A, et al: 1998. Chemokines and the arrest of lymphocytes rolling under flow conditions. Science 279: 381–384.

Chiu JJ, Usami S, Chien S: 2008. Vascular endothelial responses to altered shear stress: Pathologic implications for atherosclerosis. Ann Med 18:1–10.

Collins RG, Velji R, Guevara NV, et al: 2000. P-Selectin or intercellular adhesion molecule (ICAM)-1 deficiency substantially protects against atherosclerosis in apolipoprotein E–deficient mice. J Exp Med 191:189–194.

Combadiere C, Potteaux S, Gao JL, et al: 2003. Decreased atherosclerotic lesion formation in CX3CR1/apolipoprotein E double knockout mice. Circulation 107:1009–1016.

Combadiere C, Potteaux S, Rodero M, et al: 2008. Combined inhibition of CCL2, CX3CR1, and CCR5 abrogates Ly6C(hi) and Ly6C(lo) monocytosis and almost abolishes atherosclerosis in hypercholesterolemic mice. Circulation 117:1649–1657.

Cybulsky MI, Gimbrone MA, Jr.: 1991. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science 251:788–791.

Cybulsky MI, Iiyama K, Li H, et al: 2001. A major role for VCAM-1, but not ICAM-1, in early atherosclerosis. J Clin Invest 107: 1255–1262.

Dong ZM, Chapman SM, Brown AA, et al: 1998. The combined role of P- and E-selectins in atherosclerosis. J Clin Invest 102:145–152.

Elstad MR, La Pine TR, Cowley FS, et al: 1995. P-selectin regulates platelet-activating factor synthesis and phagocytosis by monocytes. J Immunol 155:2109–2122.

Fong AM, Robinson LA, Steeber DA, et al: 1998. Fractalkine and CX3CR1 mediate a novel mechanism of leukocyte capture, firm adhesion, and activation under physiologic flow. J Exp Med 188:1413–1419.

Galkina E, Ley K: 2007a. Leukocyte influx in atherosclerosis. Curr Drug Targets 8: 1239–1248.

Galkina E, Ley K: 2007b. Vascular adhesion molecules in atherosclerosis. Arterioscler Thromb Vasc Biol 27:2292–2301.

Gebuhrer V, Murphy JF, Bordet JC, et al: 1995. Oxidized low-density lipoprotein induces the expression of P-selectin (GMP140/PADGEM/CD62) on human endothelial cells. Biochem J 306(Pt 1):293–298.

Geissmann F, Jung S, Littman DR: 2003. Blood monocytes consist of two principal subsets with distinct migratory properties. Immunity 19:71–82.

Gerrity RG: 1981. The role of the monocyte in atherogenesis: I. Transition of blood-borne monocytes into foam cells in fatty lesions. Am J Pathol 103:181–190.

Gerszten RE, Lim YC, Ding HT, et al: 1998. Adhesion of monocytes to vascular cell adhesion molecule-1–transduced human endothelial cells: implications for atherogenesis. Circ Res 82:871–878.

Gerszten RE, Garcia-Zepeda EA, Lim YC, et al: 1999. MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. Nature 398:718–723.

Gu L, Okada Y, Clinton SK, et al: 1998. Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor–deficient mice. Mol Cell 2:275–281.

Haskell CA, Cleary MD, Charo IF: 1999. Molecular uncoupling of fractalkine-mediated cell adhesion and signal transduction. Rapid flow arrest of CX3CR1-expressing cells is independent of G-protein activation. J Biol Chem 274: 10053–10058.

Hermand P, Pincet F, Carvalho S, et al: 2008. Functional adhesiveness of the CX3CL11 chemokine requires its aggregation: role of the transmembrane domain. J Biol Chem 283:30225–30234.

Huo Y, Ley K: 2001. Adhesion molecules and atherogenesis. Acta Physiol Scand 173: 35–43.

Huo Y, Ley KF: 2004. Role of platelets in the development of atherosclerosis. Trends Cardiovasc Med 14:18–22.

Huo Y, Hafezi-Moghadam A, Ley K: 2000. Role of vascular cell adhesion molecule-1 and fibronectin connecting segment-1 in monocyte rolling and adhesion on early atherosclerotic lesions. Circ Res 87:153–159.

Huo Y, Weber C, Forlow SB, et al: 2001. The chemokine KC, but not monocyte chemoattractant protein-1, triggers monocyte arrest

on early atherosclerotic endothelium. J Clin Invest 108:1307–1314.

Huo Y, Schober A, Forlow SB, et al: 2003. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. Nat Med 9:61–67.

Imhof BA, Aurrand-Lions M: 2004. Adhesion mechanisms regulating the migration of monocytes. Nat Rev Immunol 4:432–444.

Ishibashi S, Brown MS, Goldstein JL, et al: 1993. Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. J Clin Invest 92:883–893.

Jia T, Serbina NV, Brandl K, et al: 2008. Additive roles for MCP-1 and MCP-3 in CCR2-mediated recruitment of inflammatory monocytes during *Listeria monocytogenes* infection. J Immunol 180: 6846–6853.

Khan BV, Parthasarathy SS, Alexander RW, Medford RM: 1995. Modified low density lipoprotein and its constituents augment cytokine-activated vascular cell adhesion molecule-1 gene expression in human vascular endothelial cells. J Clin Invest 95: 1262–1270.

Lesnik P, Haskell CA, Charo IF: 2003. Decreased atherosclerosis in CX3CR1–/– mice reveals a role for fractalkine in atherogenesis. J Clin Invest 111:333–340.

Ley K, Kansas GS: 2004. Selectins in T-cell recruitment to non-lymphoid tissues and sites of inflammation. Nat Rev Immunol 4: 325–335.

Lortat-Jacob H, Grosdidier A, Imberty A: 2002. Structural diversity of heparan sulfate binding domains in chemokines. Proc Natl Acad Sci U S A 99:1229–1234.

Luo BH, Carman CV, Springer TA: 2007. Structural basis of integrin regulation and signaling. Annu Rev Immunol 25: 619–647.

Luscinskas FW, Kansas GS, Ding H, et al: 1994. Monocyte rolling, arrest and spreading on IL-4–activated vascular endothelium under flow is mediated via sequential action of L-selectin, beta 1-integrins, and beta 2-integrins. J Cell Biol 125:1417–1427.

Ma YQ, Plow EF, Geng JG: 2004. P-selectin binding to P-selectin glycoprotein ligand-1 induces an intermediate state of alphaMbeta2 activation and acts cooperatively with extracellular stimuli to support maximal adhesion of human neutrophils. Blood 104: 2549–2556.

McEver RP, Moore KL, Cummings RD: 1995. Leukocyte trafficking mediated by selectin-carbohydrate interactions. J Biol Chem 270: 11025–11028.

Nageh MF, Sandberg ET, Marotti KR, et al: 1997. Deficiency of inflammatory cell adhesion molecules protects against atherosclerosis in mice. Arterioscler Thromb Vasc Biol 17:1517–1520.

Poston RN, Haskard DO, Coucher JR, et al: 1992. Expression of intercellular adhesion molecule-1 in atherosclerotic plaques. Am J Pathol 140:665–673.

Pruenster M, Rot A: 2006. Throwing light on DARC. Biochem Soc Trans 34:1005–1008.

Qiao JH, Tripathi J, Mishra NK, et al: 1997. Role of macrophage colony-stimulating factor in atherosclerosis: studies of osteopetrotic mice. Am J Pathol 150:1687–1699.

Rajavashisth T, Qiao JH, Tripathi S, et al: 1998. Heterozygous osteopetrotic (op) mutation reduces atherosclerosis in LDL receptor–deficient mice. J Clin Invest 101: 2702–2710.

Ramos CL, Huo Y, Jung U, et al: 1999. Direct demonstration of P-selectin– and VCAM-1–dependent mononuclear cell rolling in early atherosclerotic lesions of apolipoprotein E–deficient mice. Circ Res 84:1237–1244.

Rivera-Nieves J, Burcin TL, Olson TS, et al: 2006. Critical role of endothelial P-selectin glycoprotein ligand 1 in chronic murine ileitis. J Exp Med 203:907–917.

Ross R: 1993. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 362:801–809.

Saederup N, Chan L, Lira SA, Charo IF: 2008. Fractalkine deficiency markedly reduces macrophage accumulation and atherosclerotic lesion formation in CCR2–/– mice: evidence for independent chemokine functions in atherogenesis. Circulation 117: 1642–1648.

Schulz C, Schafer A, Stolla M, et al: 2007. Chemokine fractalkine mediates leukocyte recruitment to inflammatory endothelial cells in flowing whole blood: a critical role for P-selectin expressed on activated platelets. Circulation 116:764–773.

Shih PT, Brennan ML, Vora DK, et al: 1999. Blocking very late antigen-4 integrin decreases leukocyte entry and fatty streak formation in mice fed an atherogenic diet. Circ Res 84:345–351.

Smith JD, Trogan E, Ginsberg M, et al: 1995. Decreased atherosclerosis in mice deficient in both macrophage colony-stimulating factor (op) and apolipoprotein E. Proc Natl Acad Sci U S A 92:8264–8268.

Smith ML, Olson TS, Ley K: 2004. CXCR2- and E-selectin–induced neutrophil arrest during inflammation in vivo. J Exp Med 200:935–939.

Smith DF, Galkina E, Ley K, Huo Y: 2005. GRO family chemokines are specialized for monocyte arrest from flow. Am J Physiol Heart Circ Physiol 289:H1976–H1984.

Subramaniam M, Frenette PS, Saffaripour S, et al: 1996. Defects in hemostasis in P-selectin–deficient mice. Blood 87:1238–1242.

Sunderkotter C, Nikolic T, Dillon MJ, et al: 2004. Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response. J Immunol 172: 4410–4417.

Swirski FK, Libby P, Aikawa E, et al: 2007. Ly-6Chi monocytes dominate hypercholesterolemia-associated monocytosis and give rise to macrophages in atheromata. J Clin Invest 117:195–205.

Tacke F, Alvarez D, Kaplan TJ, et al: 2007. Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques. J Clin Invest 117:185–194.

van der Wal AC, Das PK, Tigges AJ, Becker AE: 1992. Adhesion molecules on the endothelium and mononuclear cells in human atherosclerotic lesions. Am J Pathol 141: 1427–1433.

Von Hundelshausen P, Weber KS, Huo Y, et al: 2001. RANTES deposition by platelets triggers monocyte arrest on inflamed and atherosclerotic endothelium. Circulation 103:1772–1777.

Von Hundelshausen P, Koenen RR, Sack M, et al: 2005. Heterophilic interactions of platelet factor 4 and RANTES promote monocyte arrest on endothelium. Blood 105:924–930.

Weber C: 2003. Novel mechanistic concepts for the control of leukocyte transmigration: specialization of integrins, chemokines, and junctional molecules. J Mol Med 81:4–19.

Weber KS, von Hundelshausen P, Clark-Lewis I, et al: 1999. Differential immobilization and hierarchical involvement of chemokines in monocyte arrest and transmigration on inflamed endothelium in shear flow. Eur J Immunol 29:700–712.

Weyrich AS, McIntyre TM, McEver RP, et al: 1995. Monocyte tethering by P-selectin regulates monocyte chemotactic protein-1 and tumor necrosis factor-alpha secretion. Signal integration and NF-kappa B translocation. J Clin Invest 95:2297–2303.

World CJ, Garin G, Berk B: 2006. Vascular shear stress and activation of inflammatory genes. Curr Atheroscler Rep 8:240–244.

Yang XP, Mattagajasingh S, Su S, et al: 2007. Fractalkine upregulates intercellular adhesion molecule-1 in endothelial cells through CX3CR1 and the Jak Stat5 pathway. Circ Res 101:1001–1008.

Zand T, Hoffman AH, Savilonis BJ, et al: 1999. Lipid deposition in rat aortas with intraluminal hemispherical plug stenosis. A morphological and biophysical study. Am J Pathol 155:85–92.

Zhang SH, Reddick RL, Piedrahita JA, Maeda N: 1992. Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. Science 258:468–471.

PII S1050-1738(08)00127-8 **TCM**