Vascular Adhesion Molecules in Atherosclerosis

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Abstract—Numerous reports document the role of vascular adhesion molecules in the development and progression of atherosclerosis. Recent novel findings in the field of adhesion molecules require an updated summary of current research. In this review, we highlight the role of vascular adhesion molecules including selectins, vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule1 (ICAM-1), PECAM-1, JAMs, and connexins in atherosclerosis. The immune system is important in atherosclerosis, and significant efforts are under way to understand the vascular adhesion molecule–dependent mechanisms of immune cell trafficking into healthy and atherosclerosis-prone arterial walls. This review focuses on the role of vascular adhesion molecules in the regulation of immune cell homing during atherosclerosis and discusses future directions that will lead to better understanding of this disease. (***Arterioscler Thromb Vasc Biol*. 2007;27:000-000.)**

Key Words: atherosclerosis ■ pathophysiology ■ lymphocyte ■ leukocyte ■ monocyte ■ macrophages ■ trafficking

Atherosclerosis results in cardiovascular death of approximately 16.7 million people around the world each year (WHO Health Report, 2003). Atherosclerosis is a chronic inflammatory process that is characterized by the formation of plaques consisting of foam cells, immune cells, vascular endothelial cells (ECs), smooth muscle cells (SMCs), platelets, extracellular matrix, and a lipid-rich core with extensive necrosis and fibrosis of surrounding tissues.[1,2] Accumulating evidence suggests the involvement of the innate and adaptive immune systems in atherosclerosis.[3–6] The first evidence that immune cells are involved in atherosclerosis came from a study that showed regional accumulation of T cells and macrophages (MΦ) in human atherosclerotic plaques.[7] Most of the T cells within the atherosclerotic plaque are effector or memory T cells[8] with a prevalence of $CD4^+$ lymphocytes expressing $\alpha\beta$T cell receptors.[9] $\gamma\delta$T cells were detected in atherosclerotic vessels, but their numbers are quite small.[8] $CD3^+$ T cells are also found within the aortic adventitia of normal/noninflamed vessels of C57BL/6 mice.[10]

There is some evidence that not only proatherogenic but also antiinflammatory players of the immune system are present within the aortas. Expression of the fork-head transcription factor Foxp-3 that is necessary for the development and function of T regulatory (Treg) cells was detected within the human atherosclerotic plaques,[11] suggesting a potential involvement of Treg in atherosclerosis. Recently discovered Th17 cells are implicated in numerous autoimmune and inflammatory conditions including multiple sclerosis, inflammatory bowel disease, and arthritis.[12] To date, there are no data indicating Th17 presence within atherosclerotic vessels. Further studies will shed light on a potential role of Th17 cells in the regulation of the immune response that accompanies atherosclerosis.

B cells are detected within the atherosclerotic adventitia,[13] and $CD22^+$ B cells are found in early and advanced atherosclerotic plaques of apolipoprotein-E–deficient ($Apoe^{-/-}$) mice.[14] Recently, it has been shown that B cells reside within the adventitia of healthy aortas and form, together with T cells, tertiary lymphoid structures on atherosclerosis induction.[10,15] Because T and B cells were found in normal/noninflamed as well as in atherosclerotic aortas, it was proposed that lymphocytes actively migrate to the aortas and likely use adhesion molecules for their trafficking.

Numerous reports indicate that monocytes play a crucial role in atherosclerosis (reviewed in[1]). In response to disturbed or oscillatory flow patterns, the recruitment of monocytes preferentially occurs at the lesser curvature of healthy aortas of C57BL/6 mice.[16] Interestingly, in parallel to this migration, accumulation of $CD68^+/CD11c^-$ macrophages (MΦ) is detected within the adventitia of the lesser curvature of the aortic arch of healthy mice.[16] The recruitment of monocytes/macrophages into the atherosclerosis-prone aortic wall has been studied in some detail (review in[17,18]), however very little is known about monocyte homing into healthy aortas. During the first stage of atherosclerosis (fatty streaks), monocytes actively accumulate within the intima and further differentiate to MΦ and dendritic cells (DCs).

There are at least 2 major subsets of monocytes. Inflammatory monocytes are $Ly6C^{high}/Gr\text{-}1^+/CCR2^+/CX3CR1^+$ and a second population, sometimes called resident monocytes, is

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Ly6C^{low}/Gr-1^{low}/ CCR2^{-}/CX3CR1^{high}.[19] A recent study suggests that the percentage of Ly6C^{high} circulating blood monocytes is elevated in atherosclerotic mice, and these Ly6C^{high} monocytes preferentially migrate into the aortic wall and become lesional macrophages.[20] Vascular DCs are detected at bifurcation and curvature sites of normal arteries[21] and throughout atherosclerotic arteries.[22,23] There is some evidence that Ly6C^{low}/CCR2^{-} blood monocytes become CD11c^{+} dendritic-like cells after entering the aortic vessel wall.[24] Detailed mechanisms underlying monocyte migration into the normal and atherosclerosis-prone vessels remain to be determined; however, it is already established that CCR5, CX3CR1, and CCR2 chemokine receptors are involved in monocyte recruitment into aortic wall.[24]

The role of vascular adhesion molecules in atherosclerosis has been reviewed before.[25–28] In this review, we will focus on the new aspects of the role of atherogenic factors in the regulation of the expression of adhesion molecules and the impact of vascular adhesion molecules on the recruitment of the immune cells into the walls of arteries.

Four Major Steps That Direct Leukocyte Recruitment

Trafficking of lymphocytes through secondary lymphoid organs and leukocyte recruitment into sites of inflammation are tightly regulated processes. Adhesion molecules and chemokines play crucial roles in these events.[29,30] There are several steps in leukocyte recruitment into vascular tissues: (1) initial selectin-dependent tethering and rolling, (2) triggering of adhesion via chemokines and their receptors or through selectin binding to P-selectin glycoprotein ligand-1 (PSGL-1),[31,32] (3) integrin-dependent adhesion and adhesion strengthening by integrin clustering, (4) transmigration across endothelium.[33,30] Details of the adhesion cascade have been reviewed[29,33,30]; therefore, this review will specifically focus on the mechanisms of the adhesion cascade that recruit immune cells into the normal or atherosclerotic aortic wall.

Selectins and Atherosclerosis

L-, P-, and E-selectins are C-type lectins that bind sialylated and fucosylated carbohydrate ligands presented by sialomucins and mediate initial capture, tethering, and rolling along endothelium.[34] L-selectin is expressed on most circulating leukocytes and mediates lymphocyte rolling in high endothelial venules (HEV) of secondary lymphoid organs and at sites of chronic inflammation, where HEV-like vessels are formed.[35] L-selectin also participates in secondary capture, defined as leukocyte capture by adherent leukocytes.[36,37] P-selectin is stored in Weibel-Palade bodies of endothelial cells and in intracellular α-granules of platelets and quickly released to the plasma membrane on endothelial cell activation.[34] P- and E-selectins are expressed in acute as well as in chronically inflamed endothelium and serve as rolling molecules for monocytes, neutrophils, effector T cells, B cells, and natural killer cells.[34] P-selectin binds PSGL-1 that is expressed by all neutrophils, monocytes, and lymphocytes.[38] E-selectin is not constitutively expressed under noninflamed conditions, but is synthesized during inflammation. E-selectin binds PSGL-1,[39] CD44,[40] E-selectin ligand-1(ESL-1)[31,41] on myeloid cells and CD43 on T-helper1 lymphocytes.[42–44]

L-Selectin

Lymphocytes constitutively home into the normal/noninflamed aortic wall[10] and reside within the adventitia.[10,15] This trafficking of T and B lymphocyte is partially dependent on L-selectin, since L-selectin (encoded by the *Sell* gene)-deficient lymphocytes show about 50% reduced migration into the aorta compared with wild-type lymphocytes.[10] Interestingly, lymphocyte migration into the atherosclerotic aorta is also regulated by L-selectin, suggesting that L-selectin ligands are constitutively expressed within the normal and atherosclerosis-prone aortic wall.[10] L-selectin ligands are carbohydrate-containing molecules. PSGL-1 is an L-selectin ligand that can support secondary capture, the process by which free flowing leukocytes interact with rolling leukocytes on the endothelium.[36,45] The involvement of L-selectin in leukocyte recruitment into the aorta may depend on its ability to mediate secondary capture through L-selectin/PSGL-1 interaction.[36] Inhibition or absence of L-selectin may prevent leukocyte secondary capture and thus decrease the flux of rolling leukocytes into the atherosclerotic vessels.

L-selectin also binds to incompletely identified endothelial ligands that are collectively named Peripheral Node Addressins (PNAd), characterized by reactivity with monoclonal antibody MECA-79.[38,46] Although lymphocyte recruitment into the aortic wall is L-selectin–dependent, aortic luminal endothelial cells and newly formed tertiary lymphoid structures do not express MECA-79, suggesting that other L-selectin ligand(s) may exist in the aortas.[10] Our understanding of the role of L-selectin in atherosclerosis is very incomplete. It is not known which ligand(s) L-selectin uses to initiate primary and secondary capture, whether activated T lymphocytes use L-selectin for rolling on the atherosclerotic endothelium, or whether the absence of L-selectin on leukocytes affects the development of atherosclerosis.

P-Selectin and E-Selectin

Low density lipoprotein (LDL) is a known risk factor for atherosclerosis.[47,2] Circulating LDL can be modified, generating oxidized LDL (oxLDL), minimally modified LDL (mmLDL), and other biologically active forms that initiate inflammatory processes.[48–50] Although the mechanisms of LDL oxidation are not completely understood, myeloperoxidase, ceruloplasmin, 15-lipoxygenase, nitric oxide synthase, NAD(P)H, and xanthine oxidase have been implicated in the generation of oxLDL.[51] Circulating and especially tissue-retained oxLDL[52] affects the inflammatory status of the endothelium. oxLDL and mmLDL but not native LDL induce P-selectin expression and monocyte adhesion to activated endothelium.[53,54] In vivo, P-selectin is detected on the atherosclerotic endothelium of active plaques but not on the normal/noninflamed endothelium.[55] Endothelial expression of P-selectin in rabbits appears after 1 week on atherogenic diet, whereas monocyte accumulation and infiltration of intimal macrophages were observed 2 weeks later.[56] HUVECs from newborns with a strong family history of myocardial infarction expressed elevated levels of basal

P-selectin.[57] These results suggest that increase of P-selectin expression may be the earliest and primary event in the initiation of atherosclerosis. To investigate the importance of P-selectin in the recruitment of monocytes to atherosclerotic lesions, an ex vivo model of isolated carotid arteries was used. In this model, monocyte rolling and attachment on the carotid endothelium from *Apoe*$^{-/-}$ mice were significantly reduced by blocking P-selectin or its leukocyte ligand, PSGL-1.[58] Thus, interaction of endothelial P-selectin with PSGL-1 expressed on monocytes plays a crucial role in the initiation of monocyte adhesion.[58] To directly demonstrate the role of P-selectin in atherosclerosis, P-selectin (encoded by the *Selp* gene)-deficient mice on C57BL/6,[59] *Apoe*$^{-/-}$,[60] and LDLR (encoded by the *Ldlr* gene)-deficient[61] background were generated. In all 3 models of atherosclerosis, *Selp*$^{-/-}$ mice showed a significant reduction in MΦ numbers in the plaques and developed smaller fatty streaks at the initial stage of atherosclerosis.

There are numerous reports suggesting that circulating activated platelets are a hallmark of cardiovascular diseases. Platelets play an active role in the deposition of proinflammatory stimuli to atherosclerotic endothelium and activation of circulating monocytes. P-selectin is expressed on platelets. Bone marrow transplantation experiments showed that mice receiving *Selp*$^{-/-}$ platelets developed smaller lesions than those receiving wild-type platelets.[62] Even more dramatic findings were obtained in a model of wire-induced artery injury in *Selp*$^{-/-}$ and *Apoe*$^{-/-}$ double knockout mice.[63] A study with P-selectin (encoded by the *Selp* gene)-deficient platelets demonstrated that platelets interact with inflamed endothelium through the binding of platelet P-selectin with an endothelial ligand.[64] During transient interaction of activated platelets with atherosclerotic endothelium, platelets deposit CCL5 to the endothelium surface that leads to elevated monocyte adhesion.[64] Moreover, platelets interact with monocytes and increase affinity and avidity of leukocyte integrins, most likely through the delivery to leukocytes of proinflammatory chemokines.[65]

E-selectin is found on ECs stimulated by inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1α,[66] or platelet factor 4 (PF4), a platelet-specific chemokine released on platelet activation.[67] E-selectin is also detected on human atherosclerosis-prone ECs and on the surface of fibrous and lipid-containing human plaques.[68] In mice, genetic deficiency of E-selectin (encoded by the *Sele* gene) leads to reduction in the lesion size although this inhibition is less than that seen for *Icam1*$^{-/-}$ and *Apoe*$^{-/-}$ or *Selp*$^{-/-}$ and *Apoe*$^{-/-}$ double knockout mice.[69] The strongest effects in the inhibition of atherosclerosis have been shown in mice with combined deficiency of E and P-selectins, showing 80% and 40% protection in the early and advanced stages of the disease, respectively.[70] These data provide evidence for an overlapping function of selectins in the regulation of atherosclerosis. A summary of the involvement of vascular adhesion molecules in atherosclerosis is shown in the Table.

Integrins in Atherosclerosis

Integrins are a family of 24 cell-surface receptors composed of 18α and 8β subunits that form $\alpha\beta$ heterodimers.[71] Integrins mediate cell-cell, cell-extracellular matrix, and cell-pathogen contact. They regulate leukocyte homing, organize the immunologic synapse, participate in costimulation, migration, and phagocytosis. Integrins rapidly change the conformation of their extracellular domain structure (inside-outside signaling)[72] and are able to cluster in response to activation.[73] In parallel, ligation of integrins leads to a signal cascade from the extracellular domain to the cytoplasm (outside-inside signaling).[72] The main integrins that participate in the regulation of leukocyte trafficking are β_2 and α_4 integrins. All leukocytes constitutively express LFA-1 (CD11a/CD18 or $\alpha_L\beta_2$),[74] a member of the β_2 subfamily of integrins. $\alpha_4\beta_1$ (VLA-4) integrin is a member of α_4 subfamily and mostly expressed on monocytes and on lymphocytes with extralymphoid homing potential.[75] LFA-1 binds 2 endothelial molecules that belong to the immunoglobulin superfamily, intercellular cell adhesion molecule-1, and -2 (ICAMs), which consist of 5 and 2 repeating extracellular Ig-like domains, respectively, a transmembrane region, and short cytoplasmic domain.[76] VLA-4 binds to another member of the immunoglobulin superfamily, VCAM-1,[77] and to the CS-1 peptide of fibronectin.[78] $\alpha_4\beta_7$ integrin binds to mucosal addressin cell adhesion molecule-1 (MAdCAM-1), an adhesion molecule expressed in gut associated lymphatic tissues.[79] It is not known whether $\alpha_4\beta_7$ integrin is relevant in atherosclerosis.

$\alpha_4\beta_1$ (VLA-4) and VCAM-1

Increased expression of adhesion molecules by the activated endothelium is a critical feature of atherosclerosis. The first evidence came from data showing that the expression of VCAM-1 is induced by arterial endothelial cells in response to accumulation of cholesterol within the intima of aortas.[80] Expression of ICAM-1 and VCAM-1 was reported in human coronary arteries.[68] Furthermore, the treatment of SMCs with TNF-α led to an increase in both VCAM-1 mRNA and cell surface expression of VCAM-1, suggesting cytokine-dependent expression of aortic VCAM-1.[81] Upregulation of endothelial VCAM-1 and ICAM-1 expression in TNFα-stimulated HUVECs and subsequent elevated adhesion of monocytes to HUVECs by aldose reductase is another possible mechanism of the regulation of adhesion molecule expression.[82]

Atherosclerosis is a focal disease affecting discrete regions of the vasculature, such as vessel curvatures and bifurcations.[83] These regions are characterized by disturbed oscillatory flow[84] that induces upregulation proinflammatory adhesion molecules such as ICAM-1 and VCAM-1.[85] An atherogenic diet also rapidly induces VCAM-1 expression in aortic endothelium in rabbit aortic organ cultures in vitro and in vivo as early as 7 days after initiation of an atherogenic diet.[86] More evidence that VCAM-1 expression is regulated by proatherogenic factors came from a study that demonstrated oxLDL-induced upregulation of VCAM-1.[87] Lipoproteins containing apolipoprotein CIII (apoCIII) increase VCAM-1 and ICAM-1 expression in ECs by activating PKCβ and NF-κB.[88] It is noteworthy that VCAM-1 upregulation is detected mainly at atherosclerosis-prone sites of the endothelium.[89] In human coronary atherosclerotic plaques,

Vascular Adhesion Molecules in Atherogenesis

Migration Step	Adhesion Molecules	Model	Effect	Reference
Tethering, rolling	L-selectin	*Sell*$^{-/-}$ vs C57BL/6 and *Apoe*$^{-/-}$ lymphocytes (adoptive transfer)	50% reduction in lymphocyte homing	Galkina et al[10]
		Sell$^{-/-}$ vs C57BL/6, *Apoe*$^{-/-}$, *Ldlr*$^{-/-}$ mice (intravital microscopy of femoral artery)	Primary and secondary capture reduced up to 60%	Eriksson et al[36]
	P-selectin	C57BL/6 and *Apoe*$^{-/-}$ mice on western diet (ex vivo model of isolated carotid artery)	Reduced rolling and attachment with anti-P-selectin or PSGL-1 Abs	Ramos et al[58]
		Selp$^{-/-}$ mice on C57BL/6, *Apoe*$^{-/-}$ or *Ldlr*$^{-/-}$ background	Reduced Mϕ numbers and lesion size at the initial stage of atherosclerosis	Nageh et al[59] Dong et al[60] Collins et al[69] Johnson et al[61]
	E-selectin	*Sele*$^{-/-}$ and *Apoe*$^{-/-}$ double knockout (DKO) mice	Slightly reduced lesion area	Collins et al[69]
		Sele$^{-/-}$ and *Selp*$^{-/-}$ and *Apoe*$^{-/-}$ triple knockout (TKO) mice	Reduction in lesion area (80% and 40% for early and advanced stages)	Dong et al[58]
Adhesion		C57BL/6 and *Apoe*$^{-/-}$ mice on western diet (ex vivo model of isolated carotid artery)	Increased rolling velocity with anti- VLA-4 or VCAM-1 Abs	Ramos et al[58]
	VCAM-1	*Vcam1*$^{D4D/D4D}$ and *Ldlr*$^{-/-}$ DKO mice on western diet vs *Apoe*$^{-/-}$ mice	Reduced lesion size by 40%	Huo et al[93] Cybulsky et al[97]
	VLA-4	*Apoe*$^{-/-}$ mice on western diet (arterial injury)	Decrease in neointimal growth and neutrophil and Mϕ recruitment with anti-VLA-4 Abs Decrease in Mϕ homing to aortas (up to 75%) with anti-α_4 Abs	Barringhaus et al[95]
		Apoe$^{-/-}$ mice (adoptive transfer)		Patel et al[96]
		Icam1$^{-/-}$ and *Ldlr*$^{-/-}$ DKO mice	No significant difference in the lesion size	Cybulsky et al[97]
	ICAM-1	*Icam1*$^{-/-}$ and *Apoe*$^{-/-}$ DKO mice (arterial injury)	No protection against plaque formation	Manka et al[103]
		Icam1$^{-/-}$ mice (on western diet)	Slightly decreased lesion size compared to C57BL/6 controls	Nageh et al[59]
		Apoe$^{-/-}$ mice (short-term adoptive transfer)	Decrease in Mϕ homing to aortas (up to 65%) with anti-ICAM-1 Abs	Patel et al[96]
	CD18	*Itgb2*$^{-/-}$ mice on western diet	Decreased lesion size compared to C57BL/6 controls	Nageh et al[59]
Transmigration	JAM-A	*Apoe*$^{-/-}$ mice on western diet (ex vivo model of isolated carotid artery)	Pretreatment with soluble JAM-A-Fc inhibited monocyte and T cell accumulation	Ostermann et al[116]
		F11r$^{-/-}$ and *Apoe*$^{-/-}$ DKO mice on western diet (wire injury of carotid arteries)	Inhibition of neointimal area with concurrent decrease in CD3$^+$ T cell and Mϕ content	Zernecke et al[119]
	JAM-C	Murine injured carotid arteries	Preincubation DCs with sJAM-C reduced DC adhesion to platelets	Langer et al[130]
	Connexin 43	*Gja1*$^{-/-}$, SMC-specific, (wire injury or vascular occlusion of carotid arteries)	Increase of neointima formation	Liao et al[138]
		Gja1$^{+/-}$ and *Ldlr*$^{-/-}$ *DKO mice, (balloon injury)*	Reduce of neointimal formation	Chadjichristos et al[137]
		Gja1$^{+/-}$ and *Ldlr*$^{-/-}$ *DKO mice on western diet*	Reduced atherosclerosis with fewer Mϕ in the lesions	Kwak et al[139]
	Connexin 37	*Gja4*$^{-/-}$ and *Ldlr*$^{-/-}$ DKO mice	Increased atherosclerosis	Wong et al[140]

elevated expression of VCAM-1 and ICAM-1 and increased numbers of plaque intimal macrophages and T cells were observed within regions of plaque neovasculazation, but less in the arterial luminal endothelium.[90] These results suggest VCAM-1– and ICAM-1–dependent recruitment of immune cells through intimal neovasculature that may participate in atherosclerosis.

Secreted phospholipases A2 (sPLA2s) play an important role in the pathophysiology of atherosclerosis (reviewed in[91]). One of the SPLA2s, the human group X enzyme, has the highest catalytic activity toward phosphatidylcholine, one of the major phospholipid species of cell membranes and LDL. LDL modified by human group X enzyme increases expression of adhesion molecules on the surface of HUVECs.[92] These results link features of metabolic syndrome and atherosclerosis with the regulatory mechanisms controlling the expression of endothelial cell adhesion molecules involved in early atherosclerosis.

The absence of shear stress and flow in some systems is different from the more complicated situation in vivo. Using isolated perfused carotid arteries from *Apoe*$^{-/-}$ mice, it has been shown that blocking endothelial VCAM-1 with Abs or treating monocytes with the connecting segment-1 (CS-1) peptide reduced adhesion by 75% and increased monocyte rolling velocity on early atherosclerotic endothelium.[93] Other evidence that CS-1 is important for monocyte recruitment come from study by Shih et al showing that treatment with mmLDL results in CS-1–dependent, but E- and P-selectin, VCAM-1–, and ICAM-1–dependent increased binding of monocytes to human aortic ECs. Thus, CS-1 serves as an alternative ligand for VLA-4 expressed by endothelium in the presence of mmLDL.[94] In a model of arterial injury in *Apoe*$^{-/-}$ mice, VLA-4 also mediates the recruitment of neutrophils and monocyte and thereby promotes neointimal growth.[95] Inhibition of VLA-4 by monoclonal antibodies directed at α_4 reduced MΦ recruitment to atherosclerotic plaques in *Apoe*$^{-/-}$ mice.[96] Interactions of VCAM-1 and VLA-4 expressed on monocytes are also involved in the regulation of monocyte recruitment[97] by the stabilization of rolling interactions and prolongation of monocyte transit time.[58] An important role of VCAM-1 in atherosclerosis was confirmed in a study using genetically modified *Vcam1*$^{D4D/D4D}$*Ldlr*$^{-/-}$ mice in which the fourth Ig domain of VCAM-1 has been disrupted.[97] As a result of this manipulation, VCAM-1 mRNA and protein levels were reduced to 8% of control, but VCAM-1 partial expression allowed incomplete rescue of the lethal phenotype of *Vcam1*$^{-/-}$ embryos.[97,98] Atherosclerosis formation was reduced in *Vcam1*$^{D4D/D4D}$ mice compared with littermate controls.[97] *Vcam1*$^{D4D/+}$ heterozygous mice on the *Apoe*$^{-/-}$ background showed a gene-dosage effect and an intermediate decrease in monocyte adhesion and fatty streak formation.[99]

ICAM-1 and β_2 Integrins

Another member of the immunoglobulin superfamily, ICAM-1, is also involved in atherosclerosis, presumably through the regulation of monocyte recruitment into atherosclerosis-prone areas. ICAM-1 expression is elevated in atherosclerosis-prone aortas and is regulated by proinflammatory stimuli. As discussed above, oxLDL induces endothelial ICAM-1 upregulation (reviewed in[100]). However, not only oxLDL but also native LDL increases the expression of ICAM-1 on HUVECs and elevates monocyte adhesion to the activated endothelium.[101] In vivo administration of native LDL into *Ldlr*$^{-/-}$-recipient mice induces ICAM-1 as well as VCAM-1 expression.[102] In agreement with in vitro studies, immunohistochemical studies of human vessels indicate that human atherosclerotic plaques contain SMCs that express ICAM-1 in response to IL-1β.[81] Pretreatment of *Apoe*$^{-/-}$ mice with Abs against ICAM-1 reduced short-term MΦ homing into atherosclerotic lesions by≈70%.[96] The absence of ICAM-1 (encoded by the *Icam1* gene, *Icam1*$^{-/-}$ mice) or CD18 (encoded by the *Itgb2* gene, *Itgb2*$^{-/-}$ mice) or both resulted in partial reduction of aortic lesion size, suggesting that ICAM-1 together with CD18 participates in the regulation of monocyte homing.[59] In a wire injury model of carotid arteries, *Icam1*$^{-/-}$ and *Apoe*$^{-/-}$ double knockout mice showed no significant reduction in lesion size,[103] suggesting that ICAM-1 is more important in spontaneous atherosclerosis than in response to injury. Activated T lymphocytes show β_1- and β_2-integrin–dependent adhesion to SMCs.[104] Nothing is known about the role of integrins in recruitment of T and B lymphocytes to the aortic wall.

Platelet Endothelial Cell Adhesion Molecule-1

Platelet endothelial cell adhesion molecule-1 (PECAM-1), also known as CD31, is a member of the immunoglobulin gene superfamily, a transmembrane glycoprotein with 6 extracellular immunoglobulin (Ig)-like domains.[105] PECAM-1 is expressed at high density at the lateral borders of ECs and at lower density on the surface of hematopoietic and immune cells, including MΦ, neutrophils, monocytes, mast cells, natural killer cells, lymphocytes, and platelets.[106] PECAM-1 gene polymorphisms and elevated soluble PECAM-1 levels are associated with severe coronary artery disease.[107] In common with other adhesion molecules, PECAM-1 has important signaling properties. Within seconds, acute onset of laminar flow stimulates phosphorylation of the PECAM-1 intracellular domain, which may promote activation of PECAM-1 in atherosclerosis-prone regions of the aortic wall.[108] PECAM-1 is a mechanosensitive molecule and serves as a member of a shear stress responsive complex in association with vascular endothelial cadherin (VE-cadherin) and vascular endothelial growth factor receptor 2 (VEGF-*R2*).[109] The important role of this complex is supported by the observation that PECAM-1 (encoded by the *Pecam1* gene)-deficient mice show less activation of inflammatory genes such as ICAM-1 in response to disturbed flow.[109] PECAM-1 expression has been detected within atherosclerosis-prone aortas on endothelial cells as well as within the neovasculazation regions of the atherosclerotic plaques.[110] *Pecam1*$^{-/-}$ and *Apoe*$^{-/-}$ mice show a significant reduction in the development of atherosclerosis compared with *Apoe*$^{-/-}$ controls (Harry B., Lansey M., Sanders J., Bruce A., Schwartz M., Ley K., unpublished data).

Junctional Adhesion Molecules

Under normal/noninflamed conditions, the vascular endothelium limits protein permeability and supports very little leukocyte adhesion and recruitment. In inflammation, the endothelium quickly provides "open gates" for leukocytes to migrate to the inflamed tissues. Transmigration and retention within tissue are regulated by ICAM-1 and VCAM-1 through the interaction of their intracellular domains with moezin and ezrin and formation of endothelial docking structures for adherent leukocytes.[111] Diapedesis can be also regulated by CD99, CD99-related antigen (CD99L2), endothelial cell-selective adhesion molecule (ESAM), and junctional adhesion molecules (JAMs), JAM-A, JAM-B, and JAM-C. JAMs are members of the immunoglobulin superfamily, which are localized to intercellular junctions of polarized endothelial and epithelial cells but can also be expressed on circulating leukocytes and platelets.[112] JAMs consist of an N-terminal signal peptide, 2 extracellular immunoglobulin-like domains, a transmembrane segment, and a cytoplasmic region.[112] These proteins participate in homophilic and heterophilic cell

interactions and thus regulate the extravasation of leukocytes into tissues.

Junctional Adhesion Molecules in Atherosclerosis

An increasing body of evidence suggests that endothelial junctional proteins regulate the processes of leukocyte transmigration through the aortic wall and thus participate in atherosclerosis.

JAM-A is expressed in leukocytes, platelets, and endothelial and epithelial cells[113,114] and participates in monocyte transmigration across ECs,[115] possibly through the ligation of LFA-1.[116] Very high levels of JAM-A were detected in atherosclerotic *Apoe*$^{-/-}$ mice and in atherosclerotic plaques of cardiovascular patients.[117] Using *Apoe*$^{-/-}$ carotid arteries perfused ex vivo, JAM-A was shown to participate in the recruitment of monocytes and T cells into arteries.[118] Deficiency of JAM-A significantly reduced neointimal lesion formation after wire injury of carotid arteries with a decrease in neointimal MΦ content and decreased luminal expression of CCL5 derived from platelets within the injured arteries.[119] These findings suggest that interactions of platelets with the aortic wall and efficient deposition of CCL5 are at least partially dependent on JAM-A engagement. In light of the increased understanding of the role of immune cells in atherosclerosis, JAM-A–expressing DCs may also play an important role in their motility and capacity to migrate to lymph nodes. The absence of JAM-A on DCs results in increased DC migration to lymph nodes and enhanced contact hypersensitivity, reflecting the capacity of JAM-A to regulate activation of adaptive immunity.[120] Therefore, it is likely that in the setting of atherosclerosis elevated expression of JAM-A could alter the migration capacity DCs within the different tissues.

JAM-B interacts with VLA-4 on T cells[121] and likely is involved in lymphocyte homing.[122] It is tempting to speculate that JAM-B might play an important role in the recruitment of T cells into the aortas through VLA-4/JAM-B dependent mechanism.

JAM-C is expressed on a subset of lymphocytes, platelets, and endothelial cells[123–125] and participates in leukocyte-endothelial interactions and mediates leukocyte-platelet and leukocyte-endothelial interactions through Mac-1.[126,123] Expression of JAM-C is weak in healthy vessels, but significantly elevated in SMCs in the neointima and in the media of human atherosclerotic vessels.[127] It is likely that JAM-C is also involved in early atherosclerotic events, because the expression of JAM-C was detected in early atherosclerotic plaques as well as in the arterial wall underlying the lesions of *Apoe*$^{-/-}$ mice. Interestingly, oxLDL induces upregulation of JAM-C expression, which may lead to JAM-C–dependent leukocyte adhesion and transmigration.[127] JAM-C also increases vascular permeability during inflammation and is involved in angiogenesis.[128] Importantly, the disruption of JAM-C function resulted in reduced retina angiogenesis in the model of hypoxia-driven retinal neovascularization,[128] suggesting possible implication of JAM-C in the processes of neovascularization in the set of advanced atherosclerosis.

The importance of platelets in thrombosis and atherosclerosis through the contribution to leukocyte adhesion to endothelium and release of multiple secretory products including inflammatory mediators and cytokines has been shown in numerous reports.[129] Recently, an important functional role of JAM-C in platelet-dependent DC recruitment into atherosclerotic wall has been discovered.[130] Preincubation of DCs with soluble JAM-C significantly reduced their adhesion to platelets. This result suggests that JAM-C may be involved in the regulation of the immune cell recruitment into atherosclerotic aortas.

Connexins

Although connexins are not adhesion molecules, they are discussed here, because they may be relevant to immune cell recruitment to atherosclerotic lesions. Connexins may regulate leukocyte trafficking into inflamed tissues.[131] Connexins form gap junctions that connect adjacent cells and permit intercellular communication.[132] Gap junctions may form between leukocytes and leukocytes, or leukocytes and endothelial cells. Three connexins (Cx), Cx37, Cx40, and Cx43, are expressed on vascular endothelial cells. Their expression is upregulated by TNF-α.[133] The first evidence of leukocyte communication via gap junctions came from dye transfer experiments that showed that lymphocytes and endothelial cells generate functional gap junction channels during extravasation.[134] Blocking of connexins with peptides results in a modest reduction in lymphocyte transmigration across an endothelial cell monolayer.[134]

Connexins in Atherosclerosis

The first evidence that atherosclerosis might involve connexins in the regulation of leukocyte migration was generated by a study showing strong expression of messenger RNA of connexins in MΦ and foam cells in human atherosclerotic carotid arteries, but not in freshly isolated blood monocytes or pure cultures of differentiated monocyte/macrophages.[135] Treatment of carotid arteries with lipoprotein-derived phospholipid oxidation products (OxPAPC) alters connexin expression with upregulation of Cx37 and Cx43 in SMCs.[136] In contrast to SMCs, endothelial cells treated with OxPAPC showed increased Cx43 expression and diminished expression of Cx37.[136] Furthermore, dye transfer between ECs and SMCs was dramatically reduced by OxPAPC. These results provide evidence that atherosclerosis-related products may actively regulate connexin expression, and thus alter the transmigration of inflammatory cells. To shed light on the role of Cx43 in the response to vascular injury, Chadjichristos et al generated heterozygous Cx43$^{+/-}$ (encoded by the *Gja1* gene) mice on the *Ldlr*$^{-/-}$ background to investigate a role of Cx43 in vascular injury.[137] *Gja1*$^{+/-}$/*Ldlr*$^{-/-}$ mice fed a high fat diet showed reduced neointimal formation with concurrent decreased MΦ accumulation in the model of balloon injury.[137] Because Cx43 is expressed on different cell types, SMC-targeted *Gja1*$^{-/-}$ (*smGja1*$^{-/-}$) mice were made to investigate the role of Cx43 specifically in SMCs.[138] In *smGja1*$^{-/-}$ mice, wire injury models enhanced neointimal formation and adventitial growth.[138] This finding contrasts with results obtained from a model of balloon injury using

smGja1$^{+/-}$/*Ldlr*$^{-/-}$ mice.[137] The difference may reflect a specific role of SMCs in sm*Gja1*$^{-/-}$ mouse model or the influence of hypercholesterolemia on the response to vascular injury.

Reduced levels of Cx43 diminished the development of atherosclerosis in the thoracoabdominal aorta and in the aortic roots of *Gja1*$^{+/-}$/*Ldlr*$^{-/-}$ mice fed a Western diet for 14 weeks.[139] Atherosclerotic plaques of *Gja1*$^{+/-}$/*Ldlr*$^{-/-}$ mice were characterized by fewer inflammatory cells and thicker fibrous caps with more collagen and SMCs, suggesting that Cx43 is not only involved in leukocyte recruitment but also in the migration of ECs and SMCs. More evidence that connexins are involved in leukocyte trafficking to atherosclerosis-prone vessels came from a study showing that Cx37 (encoded by the *Gja4* gene)-deficient and *Apoe*$^{-/-}$ double knockout mice developed more aortic lesions compared with controls.[140] Adoptive transfer experiments revealed that the absence of Cx37 on leukocytes but not on ECs resulted in elevated monocyte/macrophage recruitment.[140] Cx37-dependent ATP release may regulate monocyte adhesion.

Vasa Vasorum

Recent reports have shown that T and B cells and some MΦ reside with the aortic adventitia of C57BL/6 and *Apoe*$^{-/-}$ mice.[10,15] Moreover, adoptive transfer experiments using flow cytometry and multiphoton-microscopy suggest that lymphocytes home to the aortas mainly through vasa vasorum.[10,141,142] This observation raises a number of questions: which adhesion molecules are expressed by microvessels in vasa vasorum? How does the atherogenic environment affect the inflammatory status of the adventitia? How important are adhesion molecules expressed in the neovasculature in directing leukocyte homing from the blood? Further research is needed to fully investigate these questions.

Concluding Remarks

During the past decade, the important role of vascular adhesion molecules in atherosclerosis has been discovered and is now recognized as a critical factor in disease initiation and progression. Despite considerable progress in our understanding of the factors that regulate the expression of vascular adhesion molecules, many fundamental questions remain to be investigated. To date, it is still unclear what kinds of adhesion molecules are responsible for the homing of DCs and T and B lymphocytes into the arterial wall. Because the aortic adventitia is a major site of lymphocyte accumulation under normal and atherogenic conditions, further studies will have to examine the biology of microvessels that form the network of vasa vasorum. An important but still unanswered question is the role of adhesion molecules in the regulation of transmigration and retention of immune cells within the aortic wall and atherosclerotic plaques. Progress in this area can be expected from novel intravital imaging techniques including multiphoton microscopy.[141] Future directions also include the development of blocking agents that might inhibit or reduce harmful recruitment of specific subsets of immune cells into the aortic wall. Although this potential therapeutic approach is very promising, it should be taken into account that inhibiting leukocyte trafficking might lead to significant reduction of host defense. Therefore, it is necessary to identify a key leukocyte subset and crucial adhesion molecules that play critical roles at the different stage of atherosclerosis development for therapeutic targeting that will minimize atherosclerosis without impairing the systemic immune response.

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Disclosures

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