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Adhesion molecules and atherogenesis

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ABSTRACT

Atherosclerosis is an inflammatory disease of the vessel wall characterized by monocyte infiltration in response to pro-atherogenic factors such as oxidized lipids. Recently, the role of specific adhesion molecules in this process has been explored. The endothelium overlying atherosclerotic lesions expresses P-selectin and the shoulder regions express vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), which is also expressed on endothelium in regions not prone to plaque development. Serum levels of soluble P-selectin, ICAM-1 and VCAM-1 are elevated in patients with angina pectoris or peripheral atherosclerotic disease. Reconstituted *in vitro* systems using monocytes on cytokine-activated endothelial cells under shear flow suggested the involvement of P-selectin, L-selectin, VCAM-1, its ligand, VLA-4 integrin and CD18 integrins. Studies of monocyte adhesion in isolated perfused carotid arteries harvested from atherosclerotic (apoE–/–) mice show a predominant involvement of P-selectin and its ligand P-selectin glycoprotein-1 (PSGL-1) in rolling and of VLA-4 and VCAM-1 in firm adhesion. Consistent with these findings, apoE–/– mice that are also deficient for P-selectin show significantly reduced atherosclerotic lesion sizes and are almost completely protected from neointimal growth after vascular injury. Milder effects are also seen in the low-density lipoprotein (LDL) receptor deficient (LDLR–/–) mouse. In a high cholesterol/cholate model, a role of ICAM-1 and CD18 integrins was also shown, but this awaits confirmation in more physiologic models. Transient blockade of the VLA-4/VCAM-1 adhesion pathway by antibodies or peptides in apoE–/– or LDLR–/– mice reduced monocyte and lipid accumulation in lesions. These data suggest that P-selectin, PSGL-1, VLA-4 and VCAM-1 are the most important adhesion molecules involved in monocyte recruitment to atherosclerotic lesions.

Keywords apoE–/– mice, atherosclerosis, P-selectin, PSGL-1, VCAM-1, VLA-4.

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ATHEROSCLEROSIS – AN INFLAMMATORY DISEASE

Atherosclerosis can be viewed as a specialized type of chronic inflammation (Ross 1999). In response to atherogenic factors, mononuclear cells in blood attach, adhere and spread on the luminal surface of the arterial tree, particularly at branches and bifurcations (Glagov *et al.* 1988). These cells migrate across the endothelium and accumulate within the intima. In the presence of oxidized low-density lipoprotein (ox-LDL), monocytes are converted to activated macrophages, take up lipoprotein particles and become foam cells (Faggiotto *et al.* 1984). The formation of foam cells and their continued accumulation in the intima, accompanied by migration of smooth muscle cells from the media, lead to the first stage of the atherosclerotic lesion, the fatty streak. Without removal of atherosclerotic factors, the fatty streak will progress to the second and third phases, fibrofatty and fibrous plaque, respectively, which are characterized by recruitment of more smooth muscle cells, proliferation of extracellular matrix components, and deposition of more intra- and extracellular lipid (Ross 1999). The critical event precipitating a heart attack or an ischaemic stroke is plaque rupture. Unstable plaque prone to rupture is characterized by a thin fibrous cap, a high foam cell content, and expression of various matrix metalloproteinases (MMP). Plaque rupture is thought to be precipitated by active inflammation and mechanical plaque fissure along the shoulder regions of the lesion (Lee *et al.* 1996).

INFLAMMATION AND ADHESION MOLECULES

During inflammation, a wide variety of cell–cell interactions are important, including leucocyte–leucocyte, leucocyte–endothelium, leucocyte–vascular smooth

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muscle cell, leucocyte–extracellular matrix and leucocyte–interstitial cell interactions. The proteins mediating these interactions, adhesion molecules, belong to four major families: (1) selectins, (2) selectin ligands, (3) integrins, (4) members of the immunoglobulin family (Harlan & Liu 1992, Springer 1994). A major function of these adhesion molecules is to promote leucocyte recruitment from the vasculature into tissue through a series of events, including leucocyte rolling along the endothelial cell surface, firm adhesion and activation, and extravasation into the tissue.

Leucocyte rolling is mediated mainly by the selectins and selectin ligands (Kansas 1996). P-selectin is expressed on endothelial cells and platelets after stimulation, E-selectin is expressed on activated endothelium and L-selectin is constitutively expressed on the majority of leucocytes. These molecules are characterized by an N-terminal calcium-dependent lectin domain, an epidermal growth factor-like domain, a series of repeats with similarities to complement binding proteins, a transmembrane segment and a cytoplasmic region. P-selectin glycoprotein-1 (PSGL-1) has been described as a physiologically important selectin ligand. Other candidate selectin ligands have been proposed, but their pathophysiologic roles remain unclear. Blockade or knockout of one or more selectins or PSGL-1 significantly affects leucocyte rolling and results in dramatic decreases in leucocyte adhesion and transmigration in inflammatory models (Kansas 1996).

Leucocyte adhesion and transmigration are mediated by integrins and members of the immunoglobulin superfamily (Harlan & Liu 1992, Springer 1994). Integrins are heterodimeric molecules whose subfamilies are defined by a common β subunit non-covalently linked with various α subuits. The β_1 integrins (CD29) are also called very late antigens (VLA) because they are expressed on lymphocytes very late after activation. The leucocyte integrins share a β_2 (CD18) subunit. CD11a/CD18 ($\alpha_L\beta_2$, LFA-1) and CD11b/CD18 ($\alpha_M\beta_2$, Mac-1) are members of this subfamily relevant to mononuclear cell trafficking. The β_3 subfamily comprises the cytoadhesins, which include $\alpha_{IIb}\beta_3$ (GPIIb/IIIa) and $\alpha_V\beta_3$, a vitronectin receptor. Some integrins have a primary binding site that recognizes a short peptide like arginine-glycine-aspartic acid (RGD), a tripeptide that exists in a number of extracellular matrix proteins (Springer 1990). One of the integrins, $\alpha_4\beta_1$ (VLA-4), recognizes a motif containing the sequence glutamic acid-isoleucine-leucine-aspartic acid-valine-proline-semine-threomine (EILDVPST) within the alternatively spliced connecting segment (CS-1) region of fibronectin, with the leucine-aspartic acid-valine (LDV) sequence being most critical (Komoriya *et al.* 1991). Integrins are constitutively expressed on leucocytes, endothelial cells, as well as other cells and mediate both cell–cell and cell–matrix interactions.

The immunoglobulin superfamily members are type I transmembrane proteins characterized by a series of repeating extracellular IgG-like domains, a transmembrane region and a short cytoplasmic tail. Adhesion molecules in this family include intercellular adhesion molecules-1, 2, 3 (ICAM-1, 2 and 3), platelet endothelial cell adhesion molecule-1 (PECAM-1), and vascular cell adhesion molecule-1 (VCAM-1). The expression of VCAM-1 and ICAM-1 increases after stimulation of endothelium by inflammatory cytokines, while PECAM-1 is constitutively expressed on resting endothelial cells. VCAM-1 is an important endothelial ligand for VLA-4. ICAM-1, -2 and -3 bind to $\alpha_L\beta_2$ integrin (LFA-1), and ICAM-1 and -2 also bind to $\alpha_M\beta_2$ (Mac-1). PECAM-1 engages in homotypic adhesion to PECAM-1 molecules on other cells and may also bind to $\alpha_V\beta_3$ integrin. Blockade or loss of one or more of the members of the immunoglobulin superfamily will decrease leucocyte adhesion and extravasation and in some cases, also affect leucocyte rolling.

ADHESION MOLECULES AS RISK FACTORS FOR ATHEROSCLEROSIS

Expression of several adhesion molecules was found on established atherosclerotic lesions (Table 1). In human atherosclerotic lesions, strong expression of P-selectin was detected on the endothelium overlying active atherosclerotic plaques but not on normal arterial endothelium or on endothelium overlying inactive fibrous plaques (Johnson-Tidey *et al.* 1994). Spotty E-selectin expression was confined to endothelial cells and occurred on the surface of fibrous and lipid-containing plaques (Davies *et al.* 1993). However, E-selectin expression was not confirmed by other investigators (Iiyama *et al.* 1999). The expression of ICAM-1 was detected on endothelial cells, macrophages and smooth muscle cells of plaques with an increase after vascular injury (Manka *et al.* 1999). Normal arterial endothelial cells and intimal smooth muscle cells outside plaques gave weaker or negative reactions (Poston *et al.* 1992, Johnson-Tidey *et al.* 1994). Unlike E-selectin and ICAM-1, VCAM-1 staining of surface endothelium only occurred in fibrous and lipid-containing plaques (O'Brien *et al.* 1993). In addition, subsets of plaque smooth muscle cells and macrophages also express VCAM-1 (Davies *et al.* 1993, O'Brien *et al.* 1993), and this expression is vastly increased after injury (Manka *et al.* 1999).

Based on this profile of expression of adhesion molecules, the response-to-injury hypothesis of atherosclerosis was proposed, a theory that supposes the recruitment of mononuclear cells from the luminal

Table 1 Expression of adhesion molecules in atherosclerotic lesion

Adhesion molecules	Sample	Observation
P-selectin	Human arteries with atherosclerotic lesions	Strong P-selectin expression on the endothelium overlying active atherosclerotic plaques, not fibrous plaques (Johnson-Tidey *et al.* 1994)
	Aortas of rabbits with hypercholesterolaemia	Endothelial expression of P-selectin at 1 week, macrophages and T lymphocytes recruitment at 3 weeks (Sakai *et al.* 1997)
E-selectin	Human arteries with atherosclerotic lesions	Expression confined to endothelial cells and occurred on the surface of fibrous and lipid-containing plaques (Davies *et al.* 1993)
	Aortas of rabbits with hypercholesterolaemia	Expression confined to very few endothelial cells covering foam cell lesions (Sakai *et al.* 1997)
	Aortas of rabbits, apoE−/− or LDLR−/− mice	E-selectin mRNA and protein not detectable on arteries prone to develop or have developed atherosclerotic lesion (Iiyama *et al.* 1999)
ICAM-1	Human arteries with atherosclerotic lesions	Increased expression of ICAM-1 on endothelial layer of all lesion subtypes, on macrophages and smooth muscle cells of the plaques (Poston *et al.* 1992, Johnson-Tidey *et al.* 1994)
	Lesion-prone sites on aortas of C57BL/6 mice or rabbits	Expression on the endothelial cell surface in lesion-prone areas and other areas (Iiyama *et al.* 1999, Nakashima *et al.* 1998)
	Aortas of C57BL/6 mice or rabbits with hypercholesterolaemia	Predominant expression on endothelium in early lesions and by intimal cells in more advanced lesions; more intense staining on endothelial cells at and adjacent to lesion borders (Iiyama *et al.* 1999)
VCAM-1	Human arteries with atherosclerotic lesions	Expressed on the endothelium in fibrous and lipid-containing plaques, on SMCs and macrophages in lesion (Davies *et al.* 1993, O'Brien *et al.* 1993)
	Aortas of rabbits with hypercholesterolaemia	VCAM-1 expressed at 1 week before macrophage infiltration and sustained until the end of experiments (Sakai *et al.* 1997)
	Lesion-prone sites on aortas of C57BL/6 mice or rabbits	VCAM-1 expression increased on the endothelial cell surface in lesion-prone areas (Iiyama *et al.* 1999, Nakashima *et al.* 1998, Li *et al.* 1993)
	Atherosclerotic arteries of C57BL/6 mice or rabbits	Predominant expression on endothelium in early lesions and by intimal cells in more advanced lesions; more intense staining on endothelial cells at and adjacent to lesion borders (Iiyama *et al.* 1999)
PECAM-1	Aortas of apoE−/− mice with cholesterolaemia	Expression localized at the cell periphery throughout the aorta, no relation to lesion, not regulated (Nakashima *et al.* 1998)
Fibronectin	Human arteries with atherosclerotic lesions	Expression of FN on luminal surface but no VCAM-1 expression (Shih *et al.* 1999a, b)

surface of arteries (Ross 1999). However, in human coronary atherosclerotic plaques, expression of E-selectin, ICAM-1 and VCAM-1 on plaque neovasculature was twofold higher than on arterial luminal endothelium. Increased plaque intimal macrophage density was associated with expression of VCAM-1 on neovasculature and on non-endothelial cells. Increased plaque intimal T-lymphocyte density was associated with the presence of both ICAM-1 and VCAM-1 on neovasculature and on non-endothelial cells, suggesting that leucocyte recruitment through and/or activation of intimal neovasculature may participate in the pathogenesis of human atherosclerosis (O'Brien *et al.* 1996). However, the role of neomicrovessels in inflammatory cell recruitment to plaque remains unclear.

Expression of adhesion molecules was also detected on established lesions in animal models of atherosclerosis. Hypercholesterolaemia induced atherosclerotic lesion formation in rabbits, LDL-receptor-deficient (LDLR−/−) and apolipoprotein E-deficient (apoE−/−) mice. Immunohistochemical staining revealed that VCAM-1 and ICAM-1 were expressed predominantly by endothelium in early lesions and by intimal cells in more advanced lesions. Staining was most intense in endothelial cells at and adjacent to lesion borders (Nakashima *et al.* 1998, Iiyama *et al.* 1999).

Expression of adhesion molecules on established atherosclerotic lesions may be a cause or a consequence of mononuclear cell recruitment. To distinguish between these possibilities, adhesion molecule expression was investigated on endothelium prone to develop atherosclerotic lesions preceding mononuclear infiltration. In the arterial endothelium during the early phases of diet-induced atherogenesis in rabbits *in vivo*, the endothelium in the ascending aorta or in lesion-prone areas focally expressed P-selectin and VCAM-1 after only 1 week on the atherogenic diet and before the first appearance of intimal macrophages (Li *et al.* 1993, Sakai *et al.* 1997). In aortas of normal chow-fed wild-type mice and rabbits, VCAM-1 and ICAM-1, but not E-selectin,

were expressed by endothelial cells in regions predisposed to atherosclerotic lesion formation. *En face* confocal microscopy of the mouse ascending aorta and proximal arch demonstrated that VCAM-1 expression was increased on the endothelial cell surface in lesion-prone areas. By contrast ICAM-1 expression extended into areas protected from lesion formation (Iiyama *et al.* 1999). When mice were fed a western diet to cause hypercholesterolaemia, VCAM-1 appeared to be localized on groups of endothelial cells in lesion-prone sites in apoE−/− mice, but not control mice. Although ICAM-1 was the most prominent adhesion molecule in lesion-prone sites, its expression was not highly connected to lesion-prone sites in either apoE−/− mice or control mice. The PECAM-1 was localized at the cell periphery throughout the aorta, and its expression did not appear to be regulated (Nakashima *et al.* 1998).

Atherosclerotic risk factors can regulate the expression of adhesion molecules on endothelial cells in culture, suggesting there may be a cause-and-effect relationship between expression of adhesion molecules and atherogenesis. Hypercholesterolaemia is an important determinant of lesion formation, and LDL, in particular, is a significant atherogenic stimulus. Modifications to LDL (oxidized LDL, oxLDL, or minimally modified LDL, MM-LDL) make it more atherogenic than native LDL (Steinberg 1997). The LDL interactions with the endothelium induce VCAM-1 expression in human coronary artery (HCAEC) and pig aortic endothelial cells (PAEC) and VCAM-1 and E-selectin expression in human aortic endothelial cells (HAEC) (Allen *et al.* 1998). Treatment of HAECs with MM-LDL for 24 h caused a two to threefold increase of P-selectin protein, with little change in P-selectin surface expression. A 15-min histamine treatment of cells exposed to MM-LDL caused a 50–100% increase in P-selectin surface expression compared with cells not treated with the lipoprotein (Vora *et al.* 1997). Dose–response and time course studies demonstrated that oxLDL enhanced VCAM-1 expression induced by TNF-α by 63% in HAECs and by 45% in human umbilical vein endothelial cells (HUVECs) over unmodified LDL or control. OxLDL augmented TNF-α -induced ICAM-1 expression by 44% in HAECs. Similar results were obtained with 13-HPODE or lysophosphatidylcholine, significant components of oxLDL. These studies suggest that as long-term regulatory signals, specific oxidized fatty acid and phospholipid components of oxLDL augment the ability of vascular endothelial cells to express adhesion molecules (Khan *et al.* 1995).

Shear stress is another atherosclerotic risk factor; its crucial role in atherogenesis is suggested by the fact that lesions are first and mainly located on the bifurcations and branches of arteries. Low and oscillatory shear stresses are major features of the haemodynamic environment of sites opposite arterial flow dividers that are predisposed to atherosclerosis (Glagov *et al.* 1988). Prolonged oscillatory shear stress up-regulated expression of VCAM-1 on average ninefold relative to endothelial monolayers in static culture. ICAM-1 and E-selectin exhibited 11- and 7.5-fold increases, respectively (Chappell *et al.* 1998). Laminar flow induced a significant increase in the surface expression of ICAM-1 (Morigi *et al.* 1995), while it down-regulated the expression of VCAM-1 on the endothelium (Ando *et al.* 1995). In rabbit carotid arteries in which shear stress had been changed by surgical manipulations, *en face* immunofluorescence revealed that VCAM-1 expression was greatly increased under low shear stress. Monocytes adhered to endothelium under low shear stress; 65% of the monocytes colocalized with detectable VCAM-1 (Walpola *et al.* 1995). VCAM-1 expression increased to a lesser extent when shear stress was approximately doubled. ICAM-1 was detected even at normal shear stresses (Walpola *et al.* 1995). Other risk factors for atherosclerosis like hypertension (Mervaala *et al.* 1999), diabetes mellitus (Vlassara *et al.* 1995) and smoking (Shen *et al.* 1996) can also increase expression of adhesion molecules on endothelium and may have synergistic effects.

Endothelial and leucocyte adhesion molecules also exist as soluble molecules in the plasma, mainly generated by proteolytic cleavage or alternative splicing. The serum level of P-selectin, ICAM-1 and VCAM-1 is strongly associated with angina (Ghaisas *et al.* 1997), myocardial infarction (Kaikita *et al.* 1997) or the extent of atherosclerotic disease as measured by angiography (Saku *et al.* 1999).

INVOLVEMENT OF ADHESION MOLECULES IN MONONUCLEAR CELL/ENDOTHELIAL CELL INTERACTIONS AND IN ATHEROGENESIS

The expression pattern of adhesion molecules in space and time suggests a possible cause–effect relationship between expression of adhesion molecules, mononuclear cell/endothelium interaction and atherogenesis. Various studies have been carried out to test the hypothesis that expression of adhesion molecules and mononuclear cell adhesion may be important for mononuclear cell/endothelium interaction and for atherosclerotic lesion formation.

In an *in vitro* flow model system, freely flowing monocytes initially attached on TNF-α-activated HUVEC under flow via L- and P-selectin, whereas E-selectin was not involved (Luscinskas *et al.* 1996). VLA-4 did not support monocyte initial attachment. Once initially attached, a small number of monocytes

began rolling through a mechanism involving L-selectin, as well as VLA-4 and Mac-1 integrins, while the remaining monocytes became firmly adherent, or detached. Monocyte stable arrest and subsequent transendothelial migration occurred rapidly and efficiently through either VLA-4 or CD18 integrin adhesion pathways. It was necessary to block both VLA-4 and CD18 to block adhesion and transmigration. Transendothelial passage was also dependent on PECAM-1 (Luscinskas *et al.* 1996). In a similar system on IL-4-activated human umbilical vein endothelium, which selectively expresses VCAM-1 and an L-selectin ligand, but not E-selectin, L-selectin mediated monocyte rolling and also facilitated VLA-4 integrin-dependent arrest, whereas β_2-integrins were required for spreading of firmly attached monocytes on the endothelial cell surface but not their arrest (Luscinskas *et al.* 1994).

Treatment of HAECs with MM-LDL induces monocyte binding. This monocyte binding was not mediated by endothelial E-selectin, P-selectin, VCAM-1, or ICAM-1. Monocytic VLA-4 integrin and CS-1 domain of FN induced on the apical surface of HAEC by MM-LDL contribute to the interaction of monocytes with endothelium (Shih *et al.* 1999a, b). Transfecting the resting endothelium with adenovirus carrying VCAM-1, Gerszten *et al.* showed that VCAM-1 alone on the endothelium supported lymphocyte adhesion but not rolling and transmigration, while it was able to mediate monocyte rolling, firm adhesion and transmigration (Gerszten *et al.* 1996, 1998). These studies suggested that VCAM-1 might have a crucial role in mediating monocyte recruitment. However, more efficient binding of mononuclear cells to endothelium required activation of integrins by chemokines (Gerszten *et al.* 1999, Weber *et al.* 1999).

The above *in vitro* studies about roles of adhesion molecules in monocyte interaction with endothelium use endothelial cells cultured under various conditions that can only incompletely mimic the pathophysiological process of endothelial activation in atherosclerosis *in vivo*. To investigate the function of adhesion molecules in a model directly relevant to monocyte interaction with endothelium in atherogenesis, our lab developed a novel *ex vivo* model. Feeding apoE−/− mice with western diet for 5 weeks, the common carotid arteries developed atherosclerosis. We found expression of VCAM-1, ICAM-1, and P-selectin on the endothelium near the carotid bifurcation prior to monocyte infiltration of the intima (Ramos *et al.* 1999, Huo *et al.* 2000). Carotid arteries from apoE−/− mice were cannulated, perfused and mounted on an intravital microscope stage. Using continuous perfusion at 10–20 μL min^{-1} with physiological salt solution containing fluorescently labelled mononuclear cells, cell rolling and adhesion can be observed with stroboscopic epifluorescence video microscopy. Wall shear stress is about 3.0 dyn cm^{-2} and perfusion pressure is about 40–60 mmHg in this model. Monocyte rolling and attachment are nearly abrogated by blocking P-selectin or PSGL-1 (Ramos *et al.* 1999). Consequently, monocyte accumulation and adhesion on the early atherosclerotic endothelium were also dramatically inhibited (Huo *et al.* 2000). Effects of L-selectin on monocyte rolling and adhesion were not found in this model. Monocyte adhesion was reduced 95% by blockade of VLA-4. Blocking interaction of VLA-4 with FN decreased adhesion by only 30%. In contrast, blocking VCAM-1 on endothelium with antibody or treating monocytes with CS-1 peptide (EILDVPST) reduced adhesion by 75 or 68%, respectively. When VLA-4 or VCAM-1 was blocked, more mononuclear cells rolled on early lesions at significantly higher (approximately doubled) rolling velocities (Ramos *et al.* 1999, Huo *et al.* 2000).

To investigate involvement of adhesion molecules and the monocyte/endothelial interaction mediated by these adhesion molecules in the formation of atherosclerotic lesions, studies on lesion formation *in vivo* have been performed on atherosclerotic animal models using blocking antibodies or adhesion molecule knockout mice (Table 2). These results indicate what role each specific molecule plays in the formation of lesion, but in most cases, only an end point (lesion size) can be observed.

Using C57BL/6 mice fed a high fat, high cholesterol diet, Nageh *et al.* (1997) observed a 63% reduction in atherosclerotic fatty streaks in mice with a null mutation for P-selectin. LDLR−/− mice develop atherosclerotic lesions similar to those found in humans. When P-selectin–/– mice were intercrossed with LDLR−/− mice and fed an atherogenic diet rich in saturated fat and cholesterol, P-selectin–/– mice on diet for 8–20 weeks formed significantly smaller fatty streaks in the cusp region of the aortae than control mice. This difference was only observed in males. At 37 weeks on diet, the lesions in the LDLR−/− mice progressed to the fibrous plaque stage and were distributed throughout the entire aorta; their size or distribution was no longer dependent on P-selectin (Johnson *et al.* 1997). The P-selectin–/– mice were also intercrossed with apoE−/− mice. At 4 months of age on regular chow diet, apoE−/− P-selectin–/– mice had 3.5-fold smaller aortic sinus lesions than apoE−/− mice with intact P-selectin. These were limited to fatty streaks in the apoE−/− P-selectin–/– mice, whereas 70% of control lesions contained smooth muscle cells (Dong *et al.* 2000). Significantly, more of the aortic sinus circumference was covered by lesions in the control animals. The P-selectin genotype affected macrophage recruitment, because twice as many mononuclear cells

Table 2 Adhesion molecules relevant to atherogenesis

Adhesion molecules	Model	Observation
P-selectin	C57BL/6 mouse; high fat, high cholesterol	Aorta lesion size decreased by 63% in P-selectin–/– at 20 weeks (Nageh *et al.* 1997)
	LDLR−/− mouse; high fat, high cholesterol	Lesions in the cusp smaller in P-selectin–/– males at 8 and 20 week of diet in males, no difference in female, no difference at 37 weeks (Johnson *et al.* 1997)
	apoE−/− mouse; chow 4 or 15 months	Aortic sinus lesions reduced by 72% at 4 months, macrophage infiltration reduced by 50% in P-selectin–/– mice. At 15 months, aortic sinus lesion reduced by 62% (Dong *et al.* 2000)
	apoE−/− mouse; chow 20 weeks	Aortic lesion size in aortas reduced by 40–50% in P-selectin–/– mice (Collins *et al.* 2000)
	apoE−/− mouse; western diet and isolated carotid arteries	Rolling and adherent monocytes reduced over 80% by P-selectin mAb (Ramos *et al.* 1999)
E-selectin	apoE−/− mouse; chow 20 weeks	Aortic lesion size reduced by 25–26% in E-selectin–/– mice (Collins *et al.* 2000)
P-, E-selectin	LDLR−/− mouse; high fat, high cholesterol	Aortic lesion size decreased by 80% in P/E-selectin–/– mice at 8 weeks, after 37 week diet, by 37% and less calcification (Dong *et al.* 1998)
ICAM-1	C57BL/6 mouse; high-fat, high cholesterol	Aortic lesion size decreased by 63% in ICAM-1–/– mice at 20 weeks (Nageh *et al.* 1997)
	apoE−/− mouse; chow for 20 weeks	Aortic lesion size reduced by 31% in ICAM-1–/– mice (Collins *et al.* 2000)
	apoE−/− mouse; western diet for 40 weeks	Peritoneal macrophage homing to lesions reduced by 65% by ICAM-1 mAb (Patel *et al.* 1998)
CD18	C57BL/6 mouse; high-fat, high-cholesterol	Aortic lesion size decreased by 47% in CD18–/– (hypomorph) mice (Nageh *et al.* 1997).
ICAM-1, CD18	C57BL/6 mouse; high-fat, high-cholesterol	Aortic lesion size decreased by 76% in CD18/ICAM-1–/– mice at 20 weeks (Nageh *et al.* 1997)
VCAM-1	apoE−/− mouse; western diet and isolated carotid arteries	Adherent monocytes reduced 75% by mAb (Huo *et al.* 2000); rolling velocities doubled after VCAM-1 mAb (Ramos *et al.* 1999)
	LDLR−/− mouse; high fat	Aortic arch lesion reduced by 38% in VCAM-1 hypomorphic mutant, whole aorta lesion reduced by 48%, no effect of ICAM-1−/− (Cybulsky *et al.* 2001)
VLA-4	C57BL/6 mouse; high fat, high-cholesterol	FN CS-1 peptide reduced lesion size in aortic sinus by 68%, leucocyte infiltration reduced 48%, lipid accumulation reduced 67% (Shih *et al.* 1999a, b)
	LDLR−/− mouse; high fat, high-cholesterol	Lipid accumulation in the aortic sinus reduced by 66% (Shih *et al.* 1999a, b)
	apoE−/− mouse; western diet and isolated carotid arteries	Adherent monocytes reduced >90% by VLA-4 mAb (Huo *et al.* 2000); rolling velocities doubled after VLA-4 mAb (Ramos *et al.* 2000)
	apoE−/− mouse; western diet for 40 weeks	Peritoneal macrophages homing to lesions reduced 75% by VLA-4 mAb (Patel *et al.* 1998)

were present in the P-selectin-positive lesions. At 15 months, the lesion progressed to the fibrous plaque stage in both genotypes and spread throughout the aorta, but this process was delayed in apoE−/− P-selectin–/– mice. In the aortic sinus, the lesions of the apoE−/− P-selectin–/– mice were 2.6-fold smaller and less calcified (Dong *et al.* 2000). Collins *et al.* (2000) studied the lesion formation in apoE−/− P-selectin–/– mice and apoE−/−E-selectin–/– mice at 20 weeks of age fed a regular chow diet. Lesion area observed in P-selectin–/– mice was reduced by 40% in males, 49% in females. Therefore, P-selectin appears to be a key adhesion receptor mediating leucocyte recruitment into lesions and promoting advanced atherosclerosis in apoE−/− mice. Lesion area was also reduced in E-selectin/apoE−/− mice by 25% in males and by 26% in females (Collins *et al.* 2000). A combined effect of these selectins on the development of atherosclerotic lesions was suggested by studies in P- and E-selectin-double-deficient mice (P/E−/− mice) bred onto the LDLR−/− background (LDLR−/− P/E−/−). After 8 weeks on atherogenic diet, the LDLR−/− P/E−/− mice developed fatty streaks in the aortic sinus that were five times smaller than those in LDLR−/− P/E+/+mice. The density of macrophages in the fatty streaks was comparable between LDLR−/− P/E+/+

and LDLR−/− P/E−/− mice. After 22 weeks on the diet, the lesions spread throughout the aorta, but this process was delayed in LDLR−/− P/E−/− mice. At 37-week on diet, the lesions progressed to the fibrous plaque stage in both genotypes, the lesions in the aortic sinus in LDLR−/− P/E−/− mice were 40% smaller and less calcified than those of LDLR−/− P/E+/+ mice (Dong *et al.* 1998). However, these studies are difficult to interpret because PE−/− mice have grossly abnormal hematopoiesis, highly elevated white blood cell counts and spontaneous pathology (Bullard *et al.* 1996).

Patel *et al.* injected mouse peritoneal macrophages loaded with fluorescent microspheres intravenously into 40-week-old apoE−/− mice. After 48 h, labeled macrophages were observed adhering to all stages of atherosclerotic plaques from the early fatty streak to mature calcified lesions. Pre-treatment of the apoE−/− mice with monoclonal antibodies directed against ICAM-1 reduced macrophage homing by 65% compared with isotype-matched controls (Patel *et al.* 1998). Studies on lesion formation were also performed on female mice lacking functional ICAM-1, CD18 or both. After a high-fat, high-cholesterol diet for 20 weeks, aortic lesion size was reduced by 63% in ICAM-1–/– mice, by 47% in CD18–/– mice and by 76% in ICAM-1/CD18–/–mice (Nageh *et al.* 1997). Crossing ICAM-1–/– mice with apoE−/− mice, Collins *et al.* quantified the effect of ICAM-1 on atherosclerotic lesion formation at 20 week of age on a normal chow diet. The ICAM-1–/– mice had significantly less lesion area than their ICAM-1+/+ littermates, the lesion size decreased by 31%. The reduction in lesion area was less than that in P-selectin/apoE−/− mice but more than that in E-selectin/apoE−/− mice.

As a bridging molecule, fibrinogen can mediate leucocyte interaction with endothelium through binding $\alpha_M\beta_2$ on the leucocyte and ICAM-1 on the endothelium (Languino *et al.* 1993). To directly examine the role of fibrin(ogen) in atherogenesis, Xiao *et al.* (1998) crossed Fibrinogen-deficient mice (Fib–/–) to apoE−/− mice. They found that both apoE−/− and apoE/Fib–/– mice developed lesions throughout the entire aortic tree, ranging in appearance from simple fatty streaks to complex fibrous plaques. Furthermore, remarkably little difference in lesion size and complexity was observed within the aortas of age- and gender-matched apoE−/− and apoE/Fib–/– mice. These results indicate that the contribution of fibrin(ogen) to intimal mass and local cell adhesion, migration and proliferation is not strictly required for the development of advanced atherosclerotic disease in mice with a severe defect in lipid metabolism.

In the isolated-perfused carotid artery model, monocytes adhere to early atherosclerotic endothelium mainly through $\alpha_4\beta_1$(VLA-4) binding its endothelial ligands VCAM-1 and FN (Huo *et al.* 2000). Gene-knockout experiments showing the importance of α_4 integrins, VCAM-1 and FN for atherosclerotic lesion development have been hampered by the unavailability of these mice. Null mutations for VCAM-1 (Gurtner *et al.* 1995), α_4 (Arroyo *et al.* 1996) and FN (George *et al.* 1997), all lead to embryonic lethality so that no adult mice are available to study the impact of these molecules on atherosclerosis. However, the crucial roles of these molecules in atherosclerosis *in vivo* still can be demonstrated by peptide or antibody blocking experiments. Pretreatment of apoE−/− mice with α_4 integrin antibody reduced peritoneal macrophage homing atherosclerotic lesions by 75%, while an ICAM-1 antibody reduced it by 65% (Patel *et al.* 1998). Soluble FN CS-1 peptide interacts the VLA-4 to block binding of FN and VCAM-1. Recruitment of leucocytes in the aortic sinus of C57BL/6 mice was inhibited by 48% by chronically infusing CS-1 peptide for 24–36 h before the onset of the atherogenic diet and maintaining infusion for 4 weeks. Mice that received CS-1 peptide also demonstrated significantly reduced lesion areas (by 68%) as compared with mice that received control peptide. In a separate study, using frozen sections stained with Oil Red O to assess lipid accumulation in the aortic sinus, LDLR−/− mice receiving CS-1 peptide showed a significantly reduced area of lipid accumulation in the aortic sinus, resulting in an approximate 66% decrease. This study provides *in vivo* evidence that the VLA-4 integrin plays an important role in the initiation of the atherosclerotic lesion and lipid accumulation (Shih *et al.* 1999a, b). The important role of VCAM-1 as a ligand for VLA-4 was recently established in VCAM-1 hypomorphic mice showing 48% reduction in aortic lesion site on the LDLR−/− background (Cybulsky *et al.* 2001).

CONCLUSION

In vitro and animal studies suggest that P-selectin, PSGL-1, VLA-4 and VCAM-1 are the most important adhesion molecules in recruiting monocytes to atherosclerotic lesions. These results are supported by epidemiological evidence correlating the level of soluble adhesion molecules in serum with atherosclerotic disease. Some, but not all models suggest a role for E-selectin, ICAM-1 and β_2 integrin. Taken together, the data provide strong evidence that adhesion molecules are central to monocyte recruitment, the initiation and progression of atherosclerotic disease. Novel therapeutic approaches aimed at curbing the expression or function of adhesion molecules may prove beneficial in the management and prevention of atherosclerosis.

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