Vascular remodeling plays an important role in the development of native atherosclerosis and restenosis after PTCA. A typical atherosclerotic plaque consists of a core of lipid-rich macrophages surrounded by a thickened intima under the cover of a fibrous cap. Endothelial cell injury, related to shear forces and modified by certain risk factors, appears to be a critical initial event in atherogenesis. The surface of dysfunctional endothelium serves as a point of entry for circulating inflammatory cells, such as lymphocytes and monocytes. The recruitment of circulating monocytes and subsequent transmigration of monocytes through the dysfunctional endothelium require the careful orchestration of circulating and secreted factors and various cellular components found in the circulation and the vessel wall.

Cell adhesion molecules have emerged as essential factors in the development of atherosclerosis and restenosis. In particular, P-selectin has been established as an important marker of chronic and acute atherosclerotic events. P-selectin is present in the α-granules of inactivated platelets and in the Weibel-Palade bodies of endothelial cells. It is quickly redistributed to the surface of platelets and endothelial cells in response to activation by agonists, such as thrombin. Increased expression of P-selectin and ICAM-1 has been shown in endothelium overlying atherosclerotic plaques but not in normal arterial endothelium or in endothelium overlying inactive fibrous plaques from the carotid and coronary arteries of postmortem specimens and patients undergoing vascular surgery. Poston and Johnson-Tidey further demonstrated that anti–P-selectin antibody blocks the in vitro binding of monocytes to the endothelium but not to the denuded intima of atherosclerotic arteries, demonstrating the importance of P-selectin as a major adhesive molecule for monocyte binding to the endothelium. P-selectin has been shown to be upregulated in both acute and chronic rejection in the rat cardiac allograft arteriosclerosis model. In this model, the expression of P-selectin is correlated with the intensity of intimal thickening.

Clinically, many studies have demonstrated that elevated plasma levels of soluble P-selectin have been associated with heightened vascular activity in both acute and chronic atherosclerotic diseases. Increased plasma levels of P-selectin in patients with stable coronary artery disease before elective PTCA have been shown to correlate with both an increased risk of acute ischemic events within the first 24 hours after PTCA and an increased rate of restenosis within 6 months. The immediate surface expression of P-selectin and glycoprotein IIb/IIIa in activated platelets of blood drawn from the coronary sinus was maximal at 5 minutes after PTCA, and this expression was significantly inhibited by infusion of S-nitrosoglutathione, a potent donor of nitric oxide, 10 minutes before the PTCA. Plasma levels of P-selectin were significantly elevated after unstable angina, but were unchanged after exertional angina in patients with chronic stable coronary disease. In addition, elevated circulating levels of P-selectin have been shown to be a reliable marker of chronic atherosclerosis. Patients with chronic ischemic heart disease and peripheral vascular disease have significantly higher levels of soluble P-selectin than age- and sex-matched asymptomatic healthy control subjects. Interestingly, Karlheinz et al reported that the extent of human atherosclerosis seemed to correlate with levels of soluble cell adhesion molecule-1 but not with P-selectin, E-selectin, ICAM-1, or thrombomodulin. In addition, levels of soluble P-selectin in patients with peripheral artery disease correlated with levels of β-thromboglobulin, a marker of platelet activation, but failed to correlate with levels of either von Willebrand factor or soluble thrombomodulin, both of which are markers of endothelial cell dysfunction. In contrast, patients with chronic hypertension have been shown to have an elevation of both P-selectin and von Willebrand factor levels, and the levels correlated with elevation in diastolic pressure. This apparent difference in the pattern of markers of platelet and endothelial cell activity in peripheral vascular disease and chronic hypertension may provide important leads for future investigation, which may result in a better understanding of the interactions between various cell adhesion molecules and their pathogenic roles in chronic atherosclerotic processes.

The use of transgenic animal models has become a powerful investigative tool for a better understanding of the genetic cause of various diseases. The transgenic mouse is a particularly useful and popular model because its genome is very well characterized and can be manipulated with relative ease. The general approach is to introduce genetically engineered material into very young embryos that are subsequently reimplanted into foster mothers. The genetic material introduced in the engineered mouse can then be regulated to either overexpress or underexpress certain specific gene products at various stages of the animal’s life, and the effect on the phenotype can be studied. The use of transgenic mouse models in the study of atherosclerosis and hyperlipidemia has been well established (see review by Paigen et al). Various forms of selectin ko mice have also been generated (see review by Frenette et al.).
and Wagner15). P-selectin ko mice have a prolonged bleeding time and a major defect of leukocyte rolling, adding additional support to the concept that P-selectin may play a key role in hemostasis and in acute inflammatory response and wound repair. P-selectin has been shown to play an important role in the recruitment of lymphocytes to lymph nodes by facilitating the rolling of lymphocytes on platelet-activated endothelium.17 Double ko of P-selectin and E-selectin mice have severe leukocytosis, which is absent in single-selectin ko mice, suggesting that both selectins are important for maintaining leukocyte homeostasis. To examine the role of P-selectin in the development of fatty streaks in an LDLR-deficient mouse, which has been shown to develop an atherosclerotic lesion when fed a high-fat diet for 8 weeks, Johnson et al18 crossed a P-selectin ko mouse with an LDLR-deficient mouse. The male offspring had a twofold reduction in atherosclerotic lesion size in the cusp region of the aorta compared with P-selectin-positive mice, demonstrating the importance of P-selectin in the early development of atherosclerosis. Preliminary data from a triple ko of P-selectin, E-selectin, and LDLR demonstrated the persistent reduction of the lesion size after 22 weeks on the same high-fat diet. Thus, these transgenic studies strongly support the hypothesis that the selectins are crucial for the binding of monocytes to dysfunctional endothelium and for the binding of activated platelets to monocytes and the endothelium, two of the critical initiating events in atherogenesis.19 In spite of the abundance of valuable information gained from various studies that used transgenic mice, this model has not been as popular and successful for the study of vascular injury and restenosis because of the technical difficulty in inducing vascular injury by endothelial denudation in these small animals. Investigators have developed ingenious methods of injuring the vessels in an attempt to mimic certain aspects of vascular response after injury. Carmeliet et al19 pioneered the use of perivascular electric injury in the femoral arteries of transgenic mice. In this model, severe injury to the vessel was created by delivering an electric current to a segment of the vessel, resulting in complete destruction of the medial smooth muscle cells, denudation of the injured segment of intact endothelium, and transiently induced platelet-rich mural thrombosis. There was subsequent infiltration of inflammatory cells and progressive removal of the mural thrombus and necrotic debris after the injury. In a separate study,20 the same investigators induced the same type of injury in plasminogen-deficient mice and found that the wound healing process was significantly impaired in the plasminogen-deficient mice, with delayed removal of necrotic debris, reduced leukocyte infiltration, and smooth muscle cell migration into the necrotic center. Compared with human atherosclerosis, this model may be overly severe and physiologically irrelevant. Nevertheless, it may be a useful tool with which to study the genetic basis of vascular wound healing.

Another model of vascular injury in transgenic mice was recently reported by Kumar et al21 in which the common carotid artery was ligated at the bifurcation, resulting in significant neointimal formation in the presence of an endothelialized artery. Although this model may be criticized for being artificial and unphysiological, it provides unique insights in that there was very little thrombus formed at the site of occlusion, possibly because of the presence of an intact endothelium at the site of injury. There was an initial loss of smooth muscle cells from the media 2 days after ligation, followed by intimal hyperplasia of smooth muscle cells in the intima, with maximal response detected at 2 weeks. There was also an early inflammatory response with an increased infiltration of leukocytes in the developing intima and near the luminal surface. The inflammatory response became undetectable at 4 weeks after ligation. This model provides a unique opportunity for genetic study of the interaction between leukocytes and endothelial cells without the presence of circulating platelets, a potentially confounding factor. In a previous issue of Circulation,22 Kumar et al applied this carotid artery injury model to the study of the role of P-selectin in the early remodeling and neointimal formation of normal and P-selectin-deficient mice. The left common carotid artery was ligated in P-selectin-deficient and normal mice. The animals were killed in two groups. The first group of animals were killed at 4 weeks after ligation for evaluation of lesion formation. Significant reduction of the neointimal and medial area ratios was found in the P-selectin mice compared with the normal controls. A second group of animals were killed at 3 and 7 days after ligation for evaluation of the inflammatory leukocytes present in the developing lesions. In the P-selectin mice, there was a significant reduction of the inflammatory cells present in the developing lesions at day 7 after ligation in spite of a 49% increase of total leukocyte counts in the P-selectin mice versus normal mice. This study further affirms the importance of P-selectin in the cellular interactions between circulating leukocytes and endothelium and in the recruitment of circulating leukocytes into the vessel wall after injury.

Molecular mechanisms that underlie the sexual difference in the development of human atherosclerosis have been an important area of cardiovascular research. Emerging experimental evidence suggests a possible link between estrogen and its effect on cell adhesion molecules. In women, cyclic variation in serum estradiol levels has been associated with the maximal decrease of serum P-selectin levels in the luteal phase. A single intramuscular injection of 17β-estradiol also significantly lowered soluble P-selectin levels in healthy male volunteers, with maximal reduction occurring on day 5 after injection. Research using transgenic mice has also lent important support for the crucial role of estrogen in atherogenesis. Not unlike humans, female LDLR- and combined LDLR and P-selectin-deficient mice have recently been shown to be less susceptible to diet-induced atherosclerosis.18 Thus, the transgenic mouse appears to be an ideal model for future studies of the genetic basis of various cellular adhesion molecules in atherogenesis.
References


**KEY WORDS:** endothelium | plaque | genes
Learning From the Transgenic Mouse: Endothelium, Adhesive Molecules, and Neointimal Formation

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Circulation. 1998;97:16-18
doi: 10.1161/01.CIR.97.1.16

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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