Another Lesson From the Factor V Leiden Mouse
Thrombin Generation Drives Arterial Disease

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The discovery of activated protein C (APC) resistance by Dahlbäck in 1993 was a milestone in thrombophilia research that led to major advances in our understanding of the biochemistry, genetics, and clinical manifestations of hypercoagulability. APC resistance was quickly demonstrated to be caused by a single point mutation (1691G→A) in the coding region of the factor V gene. This mutation, factor V Leiden, is now known to be the most prevalent risk factor for venous thromboembolism (VTE) in people of European descent, occurring in 3% to 15% of the general population in Europe and North America. These observations ushered in a new era in the clinical evaluation of thrombophilia. For the first time, it became possible to diagnose a genetic defect (with a defined biochemical mechanism) in a substantial fraction of patients with venous thrombosis. Before the discovery of factor V Leiden, hereditary risk factors could be identified in <5% of patients presenting with VTE, even when a strong family history of thrombosis was obtained. In the current era, with widespread availability of genetic testing for factor V Leiden and another common hereditary risk factor, prothrombin 20210G→A, it is now possible to identify a genetic thrombophilic factor in 10% to 20% of unselected patients with VTE and up to 50% of patients with familial thrombophilia.

The factor V Leiden protein contains glutamine instead of arginine at amino acid 506, which is a key site of cleavage by APC. This substitution confers resistance to APC and leads to increased thrombin generation through 2 mechanisms: (1) decreased APC-mediated inactivation of the procoagulant form of factor V (factor Va), and (2) decreased APC-mediated conversion of factor V to an anticoagulant form (factor Vac) that functions as a cofactor for the inactivation of factor VIIIa. Thus, factor V Leiden can be considered to be both a gain-of-function mutation (leading to increased factor Va prothrombotic activity) and a loss-of-function mutation (leading to decreased factor Vac anticoagulant activity).

Many population studies have demonstrated that factor V Leiden is an independent risk factor for VTE. Estimates of the relative risk range from 2 to 7 for individuals who are heterozygous for factor V Leiden and from 20 to 80 for those who are homozygous for factor V Leiden. Factor V Leiden is also a risk factor for VTE during pregnancy and for other complications of pregnancy such as preeclampsia, placental abruption, and intrauterine fetal growth restriction. It is controversial, however, whether factor V Leiden influences the risk of arterial thrombotic disease. This question has been examined in several large prospective studies, most of which have yielded negative results. Some case-control studies have suggested that factor V Leiden may be a risk factor for stroke or myocardial infarction in certain subgroups. The strong association of factor V Leiden with venous but not arterial thrombosis is paradoxical because excessive thrombin generation might be expected to contribute importantly to thrombosis in both veins and arteries. This paradox may be related to the multifactorial nature of arterial disease, with many gene–gene and gene–environment interactions that may confound genetic association studies.

An article by Eitzman et al in this issue of Circulation sheds new light on the influence of factor V Leiden in arterial disease. Eitzman and colleagues use a murine model, the factor V Leiden mouse, to examine the effects of excessive thrombin generation on arterial thrombosis and the development of atherosclerosis. The factor V Leiden mouse carries the murine equivalent of human factor V Leiden, introduced by engineering a point mutation into the murine factor V gene. Homozygous factor V Leiden mice have the expected APC resistance phenotype and they spontaneously deposit fibrin in their tissues, a finding suggestive of chronic low-grade thrombin generation. Previous work with this murine model has provided some instructive lessons vis-à-vis the importance of genetic modifiers of thrombosis and fibrinolysis. The first lesson was that the severity of the thrombotic phenotype of the factor V Leiden mouse is highly dependent on genetic background, which implies the existence of modifier genes. A second lesson was provided by the demonstration that the factor V Leiden mouse can be used to unmask the antithrombotic phenotype of candidate genes, such as protein Z and tissue factor pathway inhibitor. These observations are being exploited by Ginsburg and colleagues, who are using the factor V Leiden/heterozygous tissue factor pathway inhibitor mouse as a platform for a mutagenesis screen to identify novel genetic modifiers of thrombosis. Another recent lesson was that the factor V Leiden mouse has impaired fibrinolytic activity, possibly because of increased activation of the thrombin-activatable fibrinolysis inhibitor. Finally, Eitzman et al report that the factor V Leiden mouse is abnormally susceptible to experimental thrombosis induced by photochemical injury of the carotid artery. This finding is consistent with reports of accelerated thrombosis in mice with other genetic abnormalities of coagulation that lead to increased thrombin generation, such as deficiency of thrombomodulin or hepatic cofactor II. Taken together, these
observations demonstrate definitively that unregulated thrombin generation can contribute to arterial thrombosis, at least in mice. Eitzman et al also used a bone marrow transplantation approach to determine that it is the plasma pool, rather than the platelet pool, of factor V Leiden that makes the greater contribution to accelerated arterial thrombosis.

An even more interesting result was obtained when Eitzman et al crossed factor V Leiden mice with apolipoprotein E (apoE)– deficient mice. ApoE-deficient mice have markedly elevated levels of plasma total cholesterol, and they develop complex atherosclerotic lesions spontaneously. At 1 year of age, apoE-deficient mice that were homozygous for factor V Leiden exhibited almost 3 times more aortic atherosclerosis than did apoE-deficient mice with wild-type factor V genes. ApoE-deficient mice that were heterozygous for factor V Leiden had an intermediate extent of atherosclerosis, which suggests that even low-grade thrombin generation may influence the development of arterial disease in mice. Importantly, all of the mice were crossed to C57BL/6 mice for several generations to minimize the influence of genetic background.

The study by Eitzman et al has some limitations. It is somewhat surprising that the potentiating effect of the factor V Leiden genotype on atherosclerosis was seen only at 1 year of age. The lack of an atherogenic effect of factor V Leiden in younger mice suggests that enhanced thrombin generation contributes to the development of late but not early atherosclerotic lesions. To solidify this conclusion, it might have been informative to perform a cross-sectional analysis of aortic lesion area in mice of different ages because the en face method used to measure the aortic surface area affected by atherosclerosis does not distinguish between early and advanced lesions. Another limitation relates to the photochemical method used to induce carotid artery thrombosis via oxidative endothelial injury. The authors suggest that this method may be a reasonable model for the type of injury that contributes to progression of vascular disease in humans, but they acknowledge that it is less relevant as a model of spontaneous complications such as myocardial infarction or stroke. It will be important, therefore, to confirm these findings via other models of arterial thrombosis. It also will be interesting to examine the susceptibility to carotid artery thrombosis of mice with both factor V Leiden and apoE deficiency because hypercholesterolemia itself may impair protein C activation and accelerate thrombosis.

The factor V Leiden mouse has proven to be a useful model of thrombotic disease, and it continues to teach us lessons about the vascular consequences of unregulated thrombin generation. The findings by Eitzman et al strongly support a role for thrombin as a driver of arterial thrombosis and atherosclerosis in mice. What can we learn from this study about the influence of factor V Leiden on arterial disease in humans? The relevance of these findings to humans who are heterozygous for factor V Leiden is uncertain and requires more study. For humans who are homozygous for factor V Leiden, however, these findings from the factor V Leiden mouse may have implications for the prevention and treatment of arterial disease. Because excessive thrombin generation appears to be particularly pathogenic during the later stages of atherosclerosis (when thrombotic complications often occur), it may be hypothesized that such patients could benefit from therapeutic intervention to inhibit thrombin or its generation, restore sensitivity to APC, or enhance fibrinolysis. This hypothesis could be tested initially in the factor V Leiden mouse and later translated to the clinical setting. As we learn more about the genetic and environmental modifiers of arterial thrombosis, similar approaches could be taken with other risk factors.

References


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