Factor V and Thrombotic Disease  
Description of a Janus-Faced Protein

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Abstract—The generation of thrombin by the prothrombinase complex constitutes an essential step in hemostasis, with thrombin being crucial for the amplification of blood coagulation, fibrin formation, and platelet activation. In the prothrombinase complex, the activated form of coagulation factor V (FVa) is an essential cofactor to the enzyme-activated factor X (FXa), FXa being virtually ineffective in the absence of its cofactor. Besides its procoagulant potential, intact factor V (FV) has an anticoagulant cofactor capacity functioning in synergy with protein S and activated protein C (APC) in APC-catalyzed inactivation of the activated form of factor VIII. The expression of anticoagulant cofactor function of FV is dependent on APC-mediated proteolysis of intact FV. Thus, FV has the potential to function in procoagulant and anticoagulant pathways, with its functional properties being modulated by proteolysis exerted by procoagulant and anticoagulant enzymes. The procoagulant enzymes factor Xa and thrombin are both able to activate circulating FV to FVa. The activity of FVa is, in turn, regulated by APC together with its cofactor protein S. In fact, the regulation of thrombin formation proceeds primarily through the upregulation and downregulation of FVa cofactor activity, and failure to control FVa activity may result in either bleeding or thrombotic complications. A prime example is APC resistance, which is the most common genetic risk factor for thrombosis. It is caused by a single point mutation in the FV gene (factor VLeiden) that not only renders FVa less susceptible to the proteolytic inactivation by APC but also impairs the anticoagulant properties of FV. This review gives a description of the dualistic character of FV and describes the gene-gene and gene-environment interactions that are important for the involvement of FV in the etiology of venous thromboembolism. (Arterioscler Thromb Vasc Biol. 2002;22:530-538.)

Key Words: factor V  ■ activated protein C resistance  ■ factor VLeiden  ■ thrombosis  ■ protein C

Historically, clinical studies focusing on coagulation factor V (FV) almost exclusively described bleeding tendencies as the result of a deficiency of this procoagulant protein. In fact, the discovery of FV by the Norwegian Paul Owren1 in 1947 was based on the identification of a patient suffering from recurrent episodes of thrombosis.5 APC resistance, which is characterized by a reduced anticoagulant response to APC, was subsequently found to be the most common risk factor for thrombosis. This review gives a description of the dualistic character of FV and describes the gene-gene and gene-environment interactions that are important for the involvement of FV in the etiology of venous thromboembolism.

Coagulation FV is an enzyme cofactor performing central and pivotal functions in maintaining a normal hemostatic balance. In the present review, we attempt to shed some light on the role that it plays in relation to the etiology of thrombotic disease.

Biosynthesis and Structure of FV
FV is a large single-chain glycoprotein of ≈330 kDa. Its plasma concentration is ≈20 nmol/L (≈0.007 g/L).11 Besides circulating in free form in plasma, FV is also present in the α-granules of platelets; this form accounts for ≈25% of the

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total FV content in human blood.11,12 During coagulation, platelet FV is secreted as a result of platelet activation. Although several cellular types have been reported to synthesize FV, it is generally accepted that the principal site of biosynthesis is the liver, where human FV is synthesized as a single-chain molecule, undergoing extensive posttranslational modifications before being secreted into the blood.13,14 It is still unclear whether the presence of FV in platelets is the result of the uptake of exogenous FV from the circulation via endocytotic processes by megakaryocytes or whether these cells themselves can account for the FV production.15–17 The FV gene (gene locus on chromosome 1q23) spans more than 80 kb and contains 25 exons. The isolated cDNA has a length of 6672 bp and encodes a preprotein of 2224 amino acids, including the 28-amino-acid residue long signal peptide.18,19 FV has a mosaic-like structure, with a domain organization (A1-A2-B-A3-C1-C2, Figure 1) that is similar to that of factor VIII (FVIII),20,21 another essential coagulation cofactor protein. The A domains of FV and FVIII together with those of ceruloplasmin22 have evolved from a common ancestral protein. Overall, the two coagulation factors (FV and FVIII) share 40% sequence identity in their A and C domains.19,23 The 3D structure of ceruloplasmin has been elucidated, and the homology between the A domains of FV and those of ceruloplasmin has allowed the creation of molecular models for the A-domain part of FV.24,25 In these models, the three A domains are arranged in a triangular fashion (Figure 1). Molecular models were also created for the C domains of FV,26 and more recently, the 3D structure of the C2 domain of FV was determined with x-ray crystallography.27 A preliminary model for the whole FVa molecule (FVa is the activated form of FV) has been generated on the basis of the information of the individual domains.24,28

Regulation of Procoagulant FXa-Cofactor Activity of FV

Circulating single-chain FV is an inactive procofactor, expressing <1% of the procoagulant enzyme-activated factor X (FXa)-cofactor activity that it can maximally obtain.29 An increase in FXa-cofactor activity is associated with limited proteolysis of several peptide bonds in FV mediated by procoagulant enzymes such as thrombin and FXa.30–32 As a result, the large connecting B domain (Figure 1) dissociates from FVa, which is formed by the noncovalently associated heavy (A1-A2) and light (A3-C1-C2) chains. The prothrombinase complex comprises FXa and FVa, which in the presence of calcium ions assemble on negatively charged phospholipid membranes. FVa is considered an essential FXa cofactor, inasmuch as its presence in the prothrombinase complex enhances the rate of prothrombin activation by several orders of magnitude.29,33 Homozygous deficiency of FV in humans is associated with variable severity of bleeding problems, suggesting that FV deficiency in humans is not a lethal disease.3 This stands in contrast to the fatal bleeding disorder that affects FV knockout mice.34 Approximately 50% of the affected embryos die during embryonic day 9 to 10, and the remaining full-term mice die from bleeding within 2 hours after birth. The explanation for the discrepancy in phenotypic severity between humans and mice is unknown.

Downregulation of the procoagulant activity of FVa is accomplished by APC-mediated proteolysis of FVa at positions Arg306, Arg506, and Arg679.35 The cleavages at these positions are under strict kinetic control, with the cleavage site at Arg506 being preferred at low concentrations of APC and FVa. However, the Arg506 cleavage yields only partial inactivation of FVa, and cleavage at Arg306 is necessary for...
the complete inactivation of FVα activity. The third cleavage at Arg679 is likely of lesser importance to FVα inactivation. Inactivation of FVα is greatly enhanced by protein S, which is an APC-cofactor protein with high affinity for negatively charged phospholipid membranes. However, the cleavages in FVα demonstrate different dependence on the APC-cofactor activity of protein S. Thus, protein S does not affect the cleavage rate at the Arg506 site, whereas the addition of protein S in systems containing purified coagulation proteins increases the rate of Arg306 cleavage 20-fold. This indicates the importance of protein S in the regulation of FVα cofactor activity. In addition, a substantial amount of evidence has been provided showing the importance of protein S for in vivo regulation of the anticoagulant protein C system, the system responsible for the proteolytic regulation of FV and FVIII. Moreover, protein S and protein C deficiencies are well-recognized risk factors for venous thrombosis, demonstrating the importance of careful regulation of FV and/or FVIII activities in vivo.

APC Resistance

In 1993, our laboratory found that plasmas from a group of patients with family histories of thromboembolic disease showed reduced anticoagulant response to the addition of APC. Because of the partial or complete resistance toward APC, we named the phenotype APC resistance and also demonstrated its inherited nature. APC resistance has been recognized as the most important cause of venous thrombosis, which is present in 20% to 60% of patients with venous thromboembolism. In the early studies, it appeared as if APC-resistant individuals were missing an essential APC cofactor. This cofactor was subsequently suggested to be intact FV. Supporting evidence for the cofactor hypothesis was the observed correction of the phenotype by added FV. On the basis of these studies, FV was proposed to act as a cofactor in the APC-catalyzed prolongation of coagulation times in plasma. The anticoagulant function of FV was further characterized later in 1994, when it was demonstrated that intact FV holds the potential to function in synergy with protein S as an APC cofactor in the inactivation of activated FVIII (FVIIa). Thus, FV, protein S, and APC regulate the activity of the tenase complex, i.e., the membrane-associated complex (comprising activated factor IX and FVIIa) that efficiently activates factor X.

The genetic background for the APC resistance phenotype was also demonstrated in 1994. A single nuclear polymorphism in the FV gene was found to be associated with APC resistance. At position 1691, a G→A missense mutation resulted in the replacement of Arg506 by Gln (FV<sub>Leiden</sub>). This mutation has an unprecedented high occurrence, with frequencies in the general population of 2% to 15% and up to 60% in selected patients with venous thromboembolism. This prevalence was 10 times higher than the sum of frequencies of all hereditary causes of thrombophilia known at that time. To date, the Arg506→Gln mutation is the most common genetic risk factor for thrombosis. Notably, variation in allelic frequencies for FV<sub>Leiden</sub> is extensive, with the mutation being present exclusively in populations of Caucasian descent. Because all FV<sub>Leiden</sub> alleles have the same haplotype, it can be concluded that the mutation occurred only once and that a founder effect has been involved. The estimated age of the mutation is ≈30 000 years; i.e., it occurred after the out-of-Africa migration that took place ≈100 000 years ago.

Because the FV Arg506→Gln mutation affects one of the prime target sites for the APC-catalyzed inactivation of FVα (see above), it appears obvious that impaired downregulation of FXa cofactor activity of FVα contributes to the increased risk of thrombosis. However, this is not the sole molecular mechanism involved, inasmuch as the mutant FV isolated from patients with APC resistance is much less active as APC cofactor in the FVIIa inactivation. This was further unequivocally demonstrated by using recombinant mutant FV. Hampered FVα inactivation alone did not satisfactorily explain the increased thrombin generation, because in vitro experiments had shown that under certain conditions (e.g., high FVα in the presence of protein S and FXa), the APC-catalyzed inactivation of normal FVα and activated FV<sub>Leiden</sub> appeared similar. The identification of the APC-cofactor function of FV reinforced the association between carriership of the FV<sub>Leiden</sub> mutation and thrombosis and contributed pathogenic explanations for the hypercoagulable state associated with APC resistance. Thus, FV presents itself as a true Janus-faced protein: In its activated form, it has essential functions in the procoagulant pathways, without which severe bleeding tendencies can occur. On the other hand, the nonactivated precursor protein factor, as it circulates in plasma, possesses anticoagulant properties functioning as an APC cofactor in the regulation of FVIIa activity. Failure to fully express this anticoagulant function may lead to thrombosis.

Anticoagulant FV

As mentioned above, an integrated account of all the functions of FV must include its anticoagulant properties as well. Still, the molecular mechanism by which FV can exert its APC-cofactor function in the downregulation of FVIIa activity is largely unknown. However, some experimental results that give an insight into the functional requirements of the anticoagulant FV function are available. Thus, full procoagulant activation of FV (i.e., cleavage at Arg709 and Arg1545 associated with the release of the B domain) results in lost anticoagulant APC-cofactor activity of FV, suggesting the involvement of the B domain in this function (Figure 1). Moreover, studies using recombinant FV variants have shown the C-terminal part of the B domain (last 70 amino acids) to be essential for the anticoagulant APC-cofactor activity of FV. The APC-mediated cleavage of intact FV at Arg506 is also involved in generating anticoagulant FV, as demonstrated with recombinant variants of FV. This explains the reduced cofactor function of FV<sub>Leiden</sub> because it cannot be cleaved at Arg506. These data indicate that a delicate balance exists between the procoagulant and anticoagulant properties of FV, with activation leading to a procoagulant protein that is devoid of anticoagulant proper-
ties (Figure 2). In contrast, APC-catalyzed proteolysis of intact FV generates an anticoagulant protein that has virtually no procoagulant properties. On the basis of discussed observations, a dual pathway determining the fate of FV in blood coagulation is emerging. In this pathway, the local concentrations and availability of procoagulant and anticoagulant enzymes, such as thrombin, FXa, and APC, determine the fate of each FV molecule. Thus, FV acts as a local sensor of procoagulant and anticoagulant forces, inasmuch as it is able to sustain ongoing reactions through its susceptibility to limited proteolysis and its ability to function as either a procoagulant or an anticoagulant cofactor.

In 1993, it was reported that human blood and platelets contain 2 forms of FV (FV\textsubscript{1} and FV\textsubscript{2}) having slightly different molecular weights and affinities for phospholipid membranes.\textsuperscript{57} The membrane-binding properties of the 2 forms affect their procoagulant and anticoagulant cofactor activities.\textsuperscript{52,58} In model systems mimicking physiological conditions, FV\textsubscript{1} appears to be the more thrombogenic, yielding up to 7-fold higher thrombin generation than FV\textsubscript{2}.\textsuperscript{58} Recently, we demonstrated the molecular difference of the FV\textsubscript{1}/FV\textsubscript{2} forms to reside in partial glycosylation of Asn2181. FV\textsubscript{1} carries a carbohydrate at this residue, in contrast to FV\textsubscript{2}, which does not.\textsuperscript{59,60}

Venous Thromboembolic Disease

Thrombosis is defined as the pathological presence of a blood clot (thrombus) in a blood vessel or the heart. Venous thrombosis and arterial thrombosis are considered distinct disease states having different pathogenic mechanisms and underlying risk factors.\textsuperscript{61–63} The estimated annual incidence of venous thromboembolism in individuals aged <40 years is 1 per 10,000, and in those aged >75 years, it is 1 per 100.\textsuperscript{63–65} Thrombosis is a complex and episodic disease, with recurrences being common. In recent years, it has become clear that venous thrombosis is multigenic\textsuperscript{66} and that the pathogenesis often includes several hereditary factors, which synergistically tip the natural hemostatic balance between procoagulant and anticoagulant forces. In addition, acquired and environmental factors modulate the risk of thrombosis and are often involved in the pathogenesis of the disease. Thrombosis is believed to develop when a certain threshold is passed as a result of risk factor interactions, with the combined total risk exceeding the sum of their separate risk contributions. Above this threshold, natural anticoagulant systems are insufficient to balance the procoagulant forces, resulting in the development of thrombotic disease.\textsuperscript{65} Most inherited risk factors for venous thrombosis are found in the protein C system, such as APC resistance (FV\textsubscript{Leiden}), and deficiencies of protein C and protein S.\textsuperscript{6,67} Other less common genetic risk factors are antithrombin deficiency and the prothrombin 20210A (PT 20210A) mutation. Acquired risk factors range from prolonged immobilization, surgery, malignant disease, trauma, use of oral contraceptives, and pregnancy/puerperium to the presence of antiphospholipid antibodies. Personal histories of thrombosis and advanced age have also been acknowledged as risk factors for venous thrombosis.\textsuperscript{68}

Role of FV\textsubscript{Leiden} in Venous Thrombosis

Thrombogenic episodes associated with FV\textsubscript{Leiden} are almost exclusively venous in nature. Although some case reports link
They Contribute

Venous Thrombosis and the Relative Risks (RR) protein S deficiency carries similar risk for thrombosis as FV Leiden and protein C deficiency (reviewed by Zöller et al69). Data adapted from Martinelli,10 Rees et al,49 Rosendaal,62 Rosendaal et al,70 Koster et al,71 and Dykes et al.72

FV Leiden to arterial thrombosis, they are rare, and these incidents can likely be due to other unrecognized pathogenic factors. A large number of studies using different approaches, such as case-control studies, population-based studies, family studies, and prospective studies, have estimated the risk increase for venous thrombosis due to FV Leiden to be 5- to 7-fold for heterozygous carriers and 80-fold for homozygous carriers of the mutation (Table 1). The severity and localization of thrombosis in carriers of FV Leiden are very diverse. Common are thromboses in the deep veins of the leg, whereas portal vein thrombosis, superficial vein thrombosis, and cerebral vein thrombosis are less prevalent. However, there seems to be no association between FV Leiden and primary pulmonary embolism73 or retinal vein thrombosis.74 Moreover, most studies do not find a higher risk of recurrent thrombosis in carriers of heterozygous FV Leiden than in other patients with thrombosis, whereas homozygous individuals have an increased risk of recurrence.75–78 The FV Leiden mutation has been suggested to be a “gain of function” mutation,62 with the overall FV levels and functions being unchanged, which distinguishes it from the “loss of function” mutations that are seen in protein C or protein S deficiency. Yet, taking the lost APC-cofactor activity of the FV Leiden molecule into account, one could still argue that this mutation induces a deficiency regarding the anticoagulant properties of FV.

The high prevalence of FV Leiden in the general white population (Table 1) related to the relatively lower annual incidence of venous thromboembolism suggests that the mutation yields a modestly increased risk of thrombosis, per se.10 However, because of the high frequency of FV Leiden in the population, combinations with other hereditary or acquired risk factors are relatively common. Because risk factors appear to synergistically increase the risk of thrombosis, many patients with thrombosis are indeed affected by >1 risk factor. For instance, the prevalence of the PT 20210A mutation is ≈2% in the general population (Table 1),79 suggesting that double mutations are present in up to 0.1% to 0.3% of white individuals. The multigenetic nature of thrombosis involving FV Leiden as a risk factor was demonstrated by the high prevalence of FV Leiden among thrombophilic families with antithrombin, protein C, or protein S deficiency or the prothrombin 20210A mutation (25%, 19%, 38%, and 10%, respectively80–84). Because these prevalences are much higher than those in the general population, it can be concluded that FV Leiden is involved in the development of thrombosis in these families. In families affected by multiple genetic risk factors, individuals having ≥2 genetic defects suffer from thrombotic events more frequently and earlier in life than do those with single defects.

One of the most common acquired risk factors associated with FV Leiden is probably the use of oral contraceptives. It is estimated that ~40% of fertile women in Sweden and the Netherlands use oral contraceptives. This suggests that many fertile women carry at least 2 risk factors of thrombosis. Synergistic effects have been shown for FV Leiden and the use of oral contraceptives, and the combined relative risk for the development of thrombosis was much higher than could be foreseen on the basis of the individual risks (Table 2; compare risk ratios). In this case, the risk factors clearly interact, although the exact molecular mechanism of this interaction still needs to be clarified. Of particular interest in this respect is the demonstration in vitro experiments that the use of oral contraceptives or of hormone replacement therapy, per se, induces an acquired resistance to APC and also that it enhances APC resistance due to FV Leiden.55,86–88 It has been debated whether investigation for APC resistance and/or FV Leiden should be performed before prescribing oral contraceptives or hormone replacement therapy, but to date, there is no consensus in favor of general screening in these situations.

Other FV Haplotypes Contributing to APC Resistance and Venous Thrombosis

Several allelic variants of the FV gene have been described. Two particularly interesting variants, FV Arg306Thr (FV Cambridge69) and FV Arg306→Gly (FV Hong Kong69), were identified in patients with thrombosis. Both these mutations result in the loss of the APC cleavage site at Arg306, which is an important cleavage site in FVa for complete loss of FVa activity (see above). FV Cambridge was identified in an individual with unexplained APC resistance and seems to be an extremely rare mutation. In contrast, FV Hong Kong is common in certain Chinese populations but does not appear to be a risk factor for thrombosis. Moreover,
FV Hong Kong is reportedly not associated with APC resistance. Intuitively, one would suspect the loss of the Arg306 cleavage site to be a risk factor of thrombosis and possibly also to yield APC resistance. In addition, the replacement of the Arg306 with a Gly and a Thr is expected to have essentially the same effect on FVα degradation. Recently, FV Cambridge and FV Hong Kong have been recreated in vitro with recombinant DNA techniques. In all functional tests, there were no appreciable differences between the 2 FV variants, with both phenotypes presenting APC responses intermediate between normal FV and FV_{Leiden}.91 Thus, even though an important APC cleavage site is lost in FV Cambridge and in FV Hong Kong, it is still not known whether these FV variants are risk factors for venous thrombosis.

Recently, several investigators have associated the so-called R2 allele (or HR2 polymorphism) with a slightly increased risk of venous thrombosis, even though no consensus has been reached regarding whether the R2 allele is really a risk factor for thrombosis.92–96 The R2 allele is characterized by several linked mutations (missense and silent) in the gene for FV. Exons 13, 16, and 25 encoding the B, A3, and C2 domains, respectively, carry these mutations.93 The R2 allele is associated with slightly decreased levels of circulating FV, which, if combined with FV_{Leiden}, may enhance the APC-resistance phenotype and increase the risk of thrombosis.94 Moreover, the plasma of carriers of the R2 allele seems to contain increased amounts of FVv,58 the form of FV that may be more thrombogenic than FV.94

Other conditions linked to the FV gene influencing APC resistance include combinations of FV_{Leiden} and quantitative FV deficiency. Heterozygous deficiency of FV combined with FV_{Leiden} results in a pseudohomozygous state of APC resistance.99–104 Because only the FV_{Leiden} allele is expressed, all circulating FV is mutated, resulting in a phenotype that is similar to that of homozygous FV_{Leiden} individuals. Taking into account the dose-response relationship between the in vitro APC response and the risk of venous thrombosis,105 it is believed that pseudohomozygous and homozygous individuals have a similar risk of thrombosis. Because pseudohomozygous APC resistance is rare, it will be difficult to find enough individuals to achieve statistical power in studies of thrombosis risk in these affected individuals. However, almost all cases of pseudohomozygous APC resistance reported to date have clinical manifestations as severe as those of homozygous individuals.

Studies have been performed to analyze whether high levels of circulating FV increase the risk of thrombosis.106 However, in contrast to reports regarding homologous FVIII, FV plasma levels did not show any statistically significant association with thrombosis. In addition, FV levels did not modify the thrombosis risk associated with high FVIII levels.

In 3 patients, spontaneously developing autoantibodies against FV were associated with the occurrence of thrombosis.107 In 1 of these patients, lupus anticoagulant activity could be detected. A second patient was found to have an elevated antiphospholipid antibody titer. The molecular mechanisms that yield the increased risk of thrombosis in the rare patients with thrombosis are not known. Nonetheless, it is possible that the autoantibodies in these patients block the anticoagulant activity of the FV molecule. This is a rare phenomenon, inasmuch as most individuals presenting with anti-FV antibodies show clinical manifestations ranging from no symptoms to life-threatening hemorrhages.107

Conclusions and Perspective

Coagulation FV is an essential cofactor protein with important functions in procoagulant and anticoagulant pathways. Regulation of FV cofactor activity is of prime importance for homeostasis of blood coagulation. Besides its activation to a procoagulant cofactor by activated coagulation enzymes such as thrombin and FXa, downregulation of FV activity under physiological conditions is the result of APC-mediated proteolysis. In contrast, proteolysis by APC of circulating intact FV recruits FV to the anticoagulant pathway because APC-cleaved intact FV functions as a synergistic cofactor with protein S in the APC-mediated regulation of FVIIIa. The most common hereditary cause of venous thrombosis is a single point mutation in FV, which results in a phenotype known as APC resistance. The FV Arg506Gln mutation (FV_{Leiden}) affects one of the target sites for APC, which results in impaired efficiency in the APC-mediated degradation of FVα. In addition, the same mutation impairs the anticoagulant APC-cofactor activity of FV in the APC-catalyzed inactivation of FVIIIa. As a result of the mutation, the regulation of thrombin formation via the protein C pathway is impaired. Although the mutation confers only a mild to moderate risk of thrombosis, it is often seen as a contributing factor in patients with thrombosis, particularly when they are affected by other genetic or acquired risk factors. Given the key importance of FV in procoagulant and anticoagulant pathways, it can be envisioned that any condition affecting FVα inactivation, on the one hand, or the expression of anticoagulant FV cofactor activity in the inactivation of FVIIIa, on the other, will increase the risk of venous thromboembolism. Such mechanisms have been suggested for the recently described association between the HR2 polymorphism and thrombosis. Because of the large size of the FV gene, it is not unlikely that within the coming years, more FV variants with altered functions and/or expression levels will be found.

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