Blood coagulation and platelet-mediated primary haemostasis have evolved as important defence mechanisms against bleeding. The coagulation system is triggered in response to rupture of endothelium, which allows exposure of blood to the extravascular tissue. The responses of the coagulation system are coordinated with the formation of the platelet plug that initially occludes the vascular lesion. Anticoagulant mechanisms ensure careful control of coagulation and, under normal conditions, they prevail over the procoagulant forces. Disturbances of the natural balance between the procoagulant and anticoagulant systems due to genetic or acquired factors may result in bleeding or thrombotic diseases.

Coagulation pathway

Thrombin is the key effector enzyme of the coagulation system, having many biologically important functions such as the activation of platelets, conversion of fibrinogen to a fibrin network, and feedback amplification of coagulation. The precise and balanced generation of thrombin at sites of vascular injury is the result of an ordered series of reactions collectively referred to as blood coagulation. The system is triggered on the surface of extravascular cells by the exposure of tissue factor to a fibrin network. Anticoagulant mechanisms ensure careful control of coagulation and, under normal conditions, they prevail over the procoagulant forces. Disturbances of the natural balance between the procoagulant and anticoagulant systems due to genetic or acquired factors may result in bleeding or thrombotic diseases.

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Tissue factor is a membrane protein abundantly present in cells surrounding the vascular bed. It binds both zymogen and activated forms of factor VII (factor VIIa). A fraction of factor VII in blood circulates as active enzyme and the binding of this form to tissue factor triggers coagulation by converting factors IX and X to their active forms (IXa and Xa). Feedback amplification is achieved when factor VII bound to tissue factor is activated by factors VIIa, IXa, and Xa. Factors IXa and Xa may remain associated with the tissue-factor-bearing cell or diffuse into the blood and bind to the surface of nearby activated platelets, which have formed the primary platelet plug. Activation of platelets is associated with the exposure of negatively charged phospholipids, which have high potential to bind coagulation factors and assemble enzyme-cofactor complexes that are crucially important for efficient propagation of the system. Prothrombin is activated to thrombin by the prothrombinase complex, which consists of the phospholipid-bound complex between the enzyme, factor Xa, and its cofactor, activated factor V (Va). The substances that activate factor V are factor Xa (on the phospholipid surface) and thrombin (both in solution and on the surface). Thrombin feedback amplifies the system by activating not only factor Va, but also factors VIII and XI. Factor VIII circulates bound to von Willebrand factor, which is an adhesive protein important for the generation of the initial platelet plug. After activation, factor VIIIa dissociates from von Willebrand factor and forms a complex on the platelet surface with factor IXa; this complex (denoted the tenase complex) then activates factor X. Activation of factor XI by thrombin is another amplification loop, resulting in the generation of additional factor IXa, which in turn activates factor X.

The initiation of coagulation via the exposure of tissue factor (tissue-factor pathway) as described above, the extrinsic pathway, is the mechanism by which coagulation is initiated in vivo in response to trauma. The intrinsic pathway is an alternative mechanism by which the coagulation system can be initiated. It involves factor XII, high-molecular-weight kininogen, prekallikrein, and factor XI; it results in the activation of factor XI. The physiological role of this pathway is not fully understood, because it is not important in trauma-initiated coagulation. Thus, inherited deficiencies of proteins of this system are not associated with bleeding problems, except for deficiency of factor XI, which leads to a moderately severe bleeding disorder.

Maximum thrombin generation occurs after the formation of the fibrin clot. This thrombin is important for additional fibrin generation as well as for activation of factor XIII and the thrombin-activatable fibrinolysis inhibitor. Activated factor XIII (XIIIa) is a transglutaminase that stabilises the clot by cross-linking of fibrin. Thrombin-activatable fibrinolysis inhibitor is a carboxypeptidase that releases carboxy-terminal lysines from fibrin. Because these lysines are important for the binding of fibrinolytic enzymes to fibrin, activation of the inhibitor prevents further fibrinolytic attack.

Phosphatidylserine is a negatively charged phospholipid required for the assembly of the tenase and prothrombinase complexes. Under normal conditions, phosphatidylserine is present in the inner-layer leaflet of the plasma cell membrane. During platelet activation, it is
The concentrations of the various coagulation proteins in circulating blood relate to their specific roles in the pathway. The predominant clotting factor is fibrinogen (10 μmol/L); this concentration is about 50 000 times higher than that of factor VIII (0·2 nmol/L). The high fibrinogen concentration is required for the formation of the fibrin network, whereas the low amount of factor VIII is more than sufficient to support factor IXa in the activation of factor X. There are also variations in the concentrations of the vitamin-K-dependent proteins: factor VII (10 nmol/L) is the least abundant, factors IX and X are present in intermediate concentrations (100 nmol/L), and prothrombin circulates at the highest concentration (2 μmol/L). Thus, early components of the pathway circulate at lower concentrations than the factors that act at later stages, which is consistent with the principal organisation of the system with multiple reactions and amplification potential. The assembly of enzyme-cofactor complexes on negatively charged phospholipid surfaces increases the local concentration of the coagulation components and counteracts regulation by anticoagulant mechanisms.

The relative importance of the various coagulation factors in vivo has been elucidated by knock-out mice technology. The crucial importance of the tissue-factor pathway is shown by the finding that mice lacking tissue factor die as embryos. Mice deficient in factor VII develop normally in utero but die shortly after birth from severe bleeding. The difference in severity between deficiency of tissue factor and deficiency of factor VII suggests a role for tissue factor during embryogenesis beyond fibrin formation. Deficiencies of factor V and prothrombin are both associated with fatal haemorrhage and partial embryonic lethality, whereas animals deficient in factor IX or VIII develop normally during fetal life, but show haemophilia-like disease after birth. As in human beings, fibrinogen deficiency in mice is associated with normal fetal development and a moderate to severe bleeding phenotype, which shows that thrombin generation is more important than fibrin deposition. This fact may be related to the multiple functions of thrombin, including its ability to activate platelets—a reaction that is crucial for the haemostasis response to vascular injury.

**Regulation of blood coagulation by anticoagulant pathways**

Regulation of coagulation is exerted at each level of the pathway, either by enzyme inhibition or by modulation of the activity of the cofactors. The tissue-factor-pathway inhibitor inhibits the reactions involving tissue factor and factor VIIa. This inhibitor is mostly bound to LDL in plasma or to heparan sulphate when associated with endothelial cells. The lack of tissue-factor-pathway inhibitor may not be compatible with life, since no deficiency states have been described in human beings.
This idea is supported by the lethal phenotype found in tissue-factor-pathway-inhibitor knock-out mice. These animals show uncontrolled activation of coagulation with consumption of coagulation factors. Most of the enzymes generated during activation of coagulation are inhibited by the serine-protease inhibitor antithrombin, previously called antithrombin III. Antithrombin preferentially inhibits free enzymes, whereas enzymes that are part of the tenase or prothrombinase complexes are less accessible for inhibition. The physiological role of antithrombin is to limit the coagulation process to sites of vascular injury and to protect the circulation from liberated enzymes. Antithrombin is, in itself, an inefficient serine-protease inhibitor, but heparin and the heparin-like molecules that are present on the surface of endothelial cells stimulate its activity. This mechanism is the molecular basis for the use of heparin as a therapeutic anticoagulant.

The protein C–anticoagulant system regulates coagulation by modulation of the activity of the two cofactors, factors VIIIa and Va. Protein C, the key component of the system, is a vitamin-K-dependent zymogen to an anticoagulant protease. It is activated on the surface of intact endothelial cells by thrombin that has bound to the membrane protein thrombomodulin. Thus, thrombin has the capacity to express both procoagulant and anticoagulant functions depending on the context under which it is generated. At sites of vascular disruption, the procoagulant effects of thrombin are fully expressed. In contrast, in an intact vascular system, thrombin has anticoagulant function since it binds to thrombomodulin and activates protein C. Activated protein C (APC) can cleave the phospholipid-membrane-bound cofactors factors Va and VIIIa, which results in inhibition of the coagulation system. A vitamin-K-dependent cofactor protein, protein S, supports the anticoagulant activity of APC. In human plasma, about 30% of protein S is free, the remainder being bound to the complement regulatory protein C4b-binding protein. APC and free protein S form a membrane-bound complex, which can cleave factors VIIIa and Va, even when they are part of fully assembled tenase and prothrombinase complexes.

In vivo, APC does not cleave intact factor VIII because the binding of factor VIII to von Willebrand factor prevents it from interacting with the phospholipid membranes. In contrast, factor V binds phospholipids as well as factor Va does, and APC is able to cleave the intact form of factor V. The consequence of APC-mediated cleavage of factor V is the generation of anticoagulant factor V that functions in synergy with protein S as an APC cofactor in the degradation of factor VIIIa. Thus, factor V can function as a procoagulant and an anticoagulant cofactor, procoagulant factor Va being formed after limited proteolysis by thrombin or factor Xa, whereas the anticoagulant factor V activity is expressed by factor V that has been proteolytically cleaved by APC. The anticoagulant potential of factor V may be particularly important in the regulation of the tenase complex by APC and protein S. The physiological importance of the protein C system is shown by the severe thromboembolic disease that is associated with homozygous deficiency of protein C in both human beings and mice. In both cases, the severe lethal thrombotic disease manifests shortly after birth. Mice lacking a functional thrombomodulin gene have even more severe disease and die during embryogenesis, even before development of a functional cardiovascular system.

Inherited and acquired coagulation disorders.

Inherited deficiencies of factor VIII (haemophilia A) and factor IX (haemophilia B) are rare inherited bleeding disorders with prevalence of about one in 10 000. The genes for both factors are on the X chromosome, which is why only males are affected, whereas females are carriers of the disease. Almost half of the disease-causing mutations are novel, and many boys with haemophilia are born in families with no previous history of the disease. Severe haemophilia is associated with less than 1% of the normal plasma concentration of either factor IX or factor VIII, whereas moderate and mild forms have plasma concentrations of 1–5% and 5–20% of normal, respectively. The normal plasma concentrations of these components seem therefore to be higher than required for a normal physiological response, which is noteworthy considering the very low normal concentrations of factor VIII in particular. The classic symptoms of haemophilia are bleeding episodes affecting joints, muscles, internal organs, and the brain. Joint bleeding (haemarthrosis) is the most characteristic feature of severe haemophilia. Repeated bleeds result in chronic arthropathy with loss of joint movement, fixed flexion contracture, and severe muscle wasting. The first bleeding manifestations appear in early childhood, but not in the neonatal period. Bleeding from the mouth caused by the eruption of the child’s teeth is a common early manifestation of haemophilia. Haemorrhages in muscles and joints after minor twists or knocks are associated with the early crawling and walking efforts. Since primary haemostasis is unaffected, the bleeding tendency of the patients do not experience major problems with bleeding from mucous membranes and minor skin lesions.

von Willebrand’s disease is caused by quantitative or qualitative defects in von Willebrand factor. This results in a primary haemostasis defect, which is caused by deficient adhesion of platelets to exposed subendothelial collagen. Bleeding from skin and mucous membranes is common, and the bleeding symptoms begin soon after birth in many cases. This disorder occurs in both male and female children. The disease is clinically heterogeneous, and the severity of the symptoms depends not only on the nature of the disease-causing mutation, but also on whether or not both alleles are affected. Three major categories of von Willebrand’s disease are distinguished. Type I refers to partial deficiency (heterozygous) and is inherited as an autosomal dominant trait. Type II (several subtypes distinguished) is associated with a qualitative defect in von Willebrand factor, which commonly affects the multimeric structure of the protein. Type III refers to total deficiency (homozygous or compound heterozygous) and is inherited as an autosomal recessive disease. Severe forms of von Willebrand’s disease have a secondary deficiency of factor VIII, since von Willebrand factor is a carrier of factor VIII in blood.

Acquired bleeding disease may be caused by an autoantibody directed against a coagulation factor, the most common being antibodies directed against factor VIII or V. Unlike inherited haemophilia, the acquired bleeding disorders mainly affect elderly people. The molecular mechanisms involved in the generation of the autoantibodies are not known. The associated bleeding tendency may be severe and occasionally life-threatening.
Another type of acquired bleeding disorder is that related to the requirement for vitamin K in the biosynthesis of many of the coagulation proteins. Conditions associated with malabsorption of vitamin K can lead to deficient γ-carboxylation of the vitamin-K-dependent coagulation proteins. In severe cases, this process results in an increased bleeding tendency. However, a more common disorder is deficiency of vitamin K due to excessive intake of antagonists, for example warfarin, used as anticoagulant therapy. Severe liver disease may be associated with bleeding tendency due to deficient synthesis of coagulation factors. The acquired bleeding tendency associated with disseminated intravascular coagulation is due to consumption of platelets and coagulation factors, which is the result of widespread pathological proteolysis. In this disorder, many proteolytic enzyme systems are activated, including both coagulation and fibrinolysis, which result in microvascular thrombosis and major disturbances of the capillary circulation. Disseminated intravascular coagulation is commonly caused by severe infections with sepsicaemia or malignant disease, but it can also complicate traumatic injury, surgery, or pregnancy.

Laboratory investigation of bleeding disorders

The initial investigation of patients with bleeding symptoms includes measurements of platelet counts, bleeding time, and global clotting tests such as the activated partial thromboplastin time (intrinsic pathway) and the prothrombin time (extrinsic or tissue-factor pathway). Patients with primary haemostatic disorders who have normal results of clotting tests but long bleeding times are further investigated with functional platelet tests, and immunological and functional assays for von Willebrand factor. When a coagulation disorder is suspected, specific functional and immunological testing of coagulation factors is possible. Severe haemophilia shows long clotting times in laboratory tests that are sensitive to the tenase complex (the activated partial thromboplastin time but not the prothrombin time, which is insensitive to the tenase complex owing to the high tissue-factor concentrations used in the assay). Specific functional assays for factors VIII and IX are used to confirm the diagnosis. Identification of causative mutations has so far been carried out only in research laboratories and has generated large databases with many different disease-generating mutations. Moderate and mild forms of haemophilia have either normal or only slightly longer than normal activated partial thromboplastin times, and the diagnosis relies on the specific tests for the respective factor. Diagnosis of acquired bleeding disorders caused by autoantibodies is based on inhibition in clotting tests by the antibodies. Identification of which specific factor is recognised by the antibody involves specific coagulation or immunological tests (eg, western blotting, which analyses the reactivity of the antibodies with various coagulation proteins). The laboratory diagnosis of vitamin K deficiency is based on the use of clotting tests examining the tissue-factor pathway (prothrombin time). The activated partial thromboplastin time is normal in most patients with this disorder. Disseminated intravascular coagulation is characterised by consumption of both platelets and coagulation factors. Thus, platelet counts are low, the activated partial thromboplastin time long, and the concentrations of fibrinogen, factor V, and factor VIII low. High concentrations of fibrin degradation products, including D-dimers, result from activation of the fibrinolytic system.

Treatment of haemophilia and von Willebrand’s disease

Concentrates of factor IX or VIII, derived either from plasma or produced by recombinant techniques, are used in the treatment of haemophilic patients. Treatment can be given on demand or as prophylaxis, techniques, are used in the treatment of haemophilic patients. Treatment can be given on demand or as prophylaxis. Drugs that inhibit platelet function, such as aspirin, should be avoided. Intramuscular injections can trigger severe bleeding episodes and should therefore not be used. In most cases, the administration of factor concentrates is uneventful but may be complicated by virus transfer (plasma-derived products) or the induction of antibody formation by the administered products. The management of haemophilic patients who have inhibitory antibodies is difficult and may include the use of high doses of factor VIIa or efforts to induce immune tolerance.3,35 Gene therapy has not so far been established as a therapeutic modality, although research in this area is very intense. In animals, gene therapy of factor IX in dogs has shown promising results.

Bleeding episodes in patients with severe forms of von Willebrand’s disease are treated with concentrates of von Willebrand factor. DDAVP (D-amino-D-arginine vasopressin) increases the release of von Willebrand factor from endothelial cells. It is therefore useful in the treatment of type I von Willebrand’s disease. However, because an efficient response to DDAVP requires that the von Willebrand factor can be synthesised, it is not effective in type III von Willebrand’s disease. Type II shows variation in the response to DDAVP, which should therefore only be used after individual testing.

Inherited and acquired thrombotic disorders

Venous thrombosis is common, each year affecting one in 1000 individuals, with higher rates among elderly people than in the young. Pulmonary embolism or post-thrombotic syndrome may complicate the disease, but in most cases the recovery is uneventful. Inherited and acquired risk factors are involved in the pathogenesis of thrombosis. The inherited risk factors are life-long, whereas most acquired risk factors are of short duration (eg, pregnancy, surgery, and immobilisation). An acquired risk factor may seem to be the cause of a thrombotic episode that is in fact due to a combination of genetic and acquired risk factors.38 Most inherited risk factors for thrombosis affect the natural balance between procoagulant and anticoagulant forces, and most are found in the protein C system.39 The most common inherited risk factor is a single point mutation in the gene for factor V (G1691A), which results in phenotype called APC resistance, found in 20–40% of patients with thrombosis.40–42 The factor V mutation predicts the replacement of arginine 506 with a glutamine residue, which results in the loss of one of the APC cleavage sites in factor V:Va.43 Mutant factor V (VR506Q, V:Q506, or factor V Leiden) has full procoagulant capacity. Dual mechanisms cause the hypercoagulable condition that characterises APC resistance.44 The factor V mutation is associated with impaired degradation of factor Va by APC, since the arginine 506 site is one of three APC cleavage sites in factor Va. In addition, the factor V mutation affects factor VIIIa degradation, because the

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anticoagulant activity of factor V is stimulated by the arginine 506 cleavage. The factor V mutation is found predominantly in populations of caucasian origin, which is explained by a single mutational event that took place around 30 000 years ago and a subsequent founder effect. The prevalence of the factor V mutation varies between different countries of Europe and, with some exceptions, a north-south gradient is apparent with highest prevalence (10–15%) in the north and lowest in the south (about 2%). In populations with mixed ethnic backgrounds, such as the USA, the prevalence is about 5%. APC resistance due to the factor V mutation is associated with a slightly increased risk of thrombosis (five to ten fold) in its heterozygous state and a greatly increased risk in the homozygous state (50–100 fold). On the other hand, APC-resistant women have a reduced bleeding tendency after delivery, which, during evolution, may have provided a survival advantage explaining the high prevalence of the mutation.

The second most common genetic risk factor for thrombosis (found in 6–8% of patients with thrombosis) is a single mutation (G20210A) in the 3’ untranslated region of the prothrombin gene. The mutation does not affect prothrombin function but is associated with slightly increased concentrations of prothrombin in plasma. It is found in around 2% of white people and is associated with slightly increased risk of thrombosis (three to five fold). Heterozygous deficiency of protein C, protein S, or antithrombin also increases the risk of thrombosis (found in 1–3% of thrombosis patients), but these deficiencies are uncommon in the general population (protein C and protein S deficiency in about one in 300 and antithrombin deficiency in one in 2000). Many different mutations in each of these genes cause the deficiency states. The risk of thrombosis in deficiency of protein C or protein S is similar to that in APC resistance, whereas antithrombin deficiency is a somewhat stronger risk factor. Since the risk of thrombosis associated with the inherited disorders is low, most individuals with a single genetic risk factor will not have thrombosis. Individuals with more than one risk factor, either genetic or acquired, have a higher risk. Venous thromboembolism is now considered to be a typical multigenetic/multifactorial disease.

The antiphospholipid syndrome (lupus anticoagulant) is an acquired risk factor for thrombosis, which can be of long duration and can cause both arterial and venous thrombosis. The antibodies in the plasma of patients with this syndrome are directed against a protein-lipid thrombosis. The antibodies in the plasma of patients with long duration and can cause both arterial and venous risk factor, either genetic or acquired, have a higher risk.

Venous thromboembolism is considered to be a typical multigenetic/multifactorial disease.

Laboratory investigation of risk factors for thrombosis

Simple functional clotting-based APC resistance tests are available with close to 100% sensitivity and specificity for the factor V mutation. Positive results are generally confirmed by DNA-based tests, which distinguish heterozygous from homozygous forms. In rare cases, the APC resistance test suggests a more severe phenotype than the DNA test, which may be due to pseudohomozygosity with one mutant factor V allele and one null allele. In this condition, all factor V molecules in the blood are APC resistant, even though DNA analysis suggests heterozygosity. Diagnosis of the prothrombin mutation (G20210A) relies on DNA testing, whereas deficiencies of protein C, protein S, and antithrombin are best diagnosed by functional or immunological assays. For protein S, assays for the free form of the protein are preferable to those measuring the total protein S, since they have higher predictive value for deficiency. Global clotting tests, such as the activated partial thromboplastin time, are useful for the initial identification of patients with antiphospholipid syndrome: in many cases of this disorder the clotting times in all tests are long because the antibodies disturb the interaction between the coagulation factors and the phospholipids. Further investigation of a lupus anticoagulant includes the use of other clotting tests, such as the dilute Russell’s viper venom time, a test in which a prothrombinase complex is generated due to activation of factors X and V by the venom. Demonstration of inhibitory activity in mixtures between normal plasma and the patient’s plasma is used to assess the potency of the lupus anticoagulants. The laboratory investigation may also include neutralisation tests with excess phospholipids and immunological tests that measure the binding of the patient’s antibodies to immobilised phospholipids.

**Treatment of patients with thrombosis**

Venous thrombosis is initially treated with a combination of heparin and vitamin K antagonists. Either unfractionated or low-molecular-weight heparin can be used; the latter is prepared from unfractionated heparin by chemical or enzymic cleavage methods. Low-molecular-weight heparin has better pharmacokinetic properties than unfractionated heparin, and adequate haemostatic control is achieved with a single daily subcutaneous injection. In addition, laboratory monitoring is not needed with low-molecular-weight heparin. After a few days of the combined treatment, the concentrations of functional vitamin-K-dependent coagulation proteins fall into the therapeutic range, and heparin is discontinued. Treatment with vitamin K antagonists, which should be regularly monitored by prothrombin time (international normalised ratio), is generally continued for 3–6 months. The risk of bleeding complications must always be weighed against the benefits of the anticoagulation effect, especially if an oral anticoagulant is used for periods exceeding 3–6 months, when the risk of thromboembolic recurrence probably declines. Whether the presence of a genetic risk factor is associated with an increased risk of recurrence is not known, though several studies of APC resistance suggest this association. Patients with combined genetic defects, and probably also patients with single gene defects, may be at increased risk of recurrence, and long-term anticoagulation beyond 6 months can be considered, even after an isolated thromboembolic event. Prophylactic treatment with low-molecular-weight heparin or oral anticoagulants is recommended for individuals with multiple genetic defects (including homozygous APC resistance) in situations known to be associated with a high risk of thromboembolic complications. This recommendation holds even if the patient neither has experienced thrombosis nor has any family history of such complications. In symptom-free carriers of single genetic risk factors lacking a family history of thrombosis, short-term prophylaxis may be considered in high-risk situations.
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