Prethrombotic states are pathologic conditions in which blood coagulation, platelet function, or fibrinolysis have been altered to favor thrombosis. Epidemiologic studies suggest that prethrombotic states may occur in some patients with stroke who have atherosclerotic risk factors, but the details of these relationships remain poorly understood. Recently, many of the natural inhibitors of thrombosis have been elucidated. In particular, the relevance of immune mechanisms in the genesis of prethrombotic states has been indicated by numerous reports linking circulating antiphospholipid antibodies (APA) to brain infarction, transient ischemic attacks (TIA), transient monocular blindness (TMB), vascular dementia, and a miscellany of other neurologic syndromes including migraine headaches.

In prethrombotic states the hemostatic equilibrium has been shifted to favor thrombosis. Since the plasma concentrations of the coagulation factors are normally present in excess, increases in the concentration of circulating nonactivated clotting factors appear unlikely to contribute to a prethrombotic state. Although epidemiologic studies suggest that high concentrations of fibrinogen, excess factor VIII coagulant activity (VIII:C), and factor VII may be risk factors for stroke, a more critical event in the formation of a thrombus is the activation of clotting factors by tissue or vascular wall injury. As illustrated in the upper portion of Figure 1, the intrinsic and extrinsic coagulation systems promote the conversion of prothrombin to thrombin which cleaves fibrinogen, producing fibrin monomer. Thrombin also activates platelets and factors V and VIII.

Considerable evidence supports the notion that a prethrombotic state is present immediately after an atherothrombotic or cardioembolic stroke, but whether activation of coagulation precedes these strokes is not established. Landi and colleagues contended that the prethrombotic state in patients following stroke is reflected by excessive thrombin activation. This argument is buttressed by a report by Feinberg et al of the relationship in stroke patients between thrombin induced fibrin formation (fibrinopeptide A) and fibrin breakdown (B-β 1-42 peptide, cross-linked D-dimer) within 4 weeks of acute brain infarction. They found that thrombin activation, which occurred immediately after stroke, continued for about 1 month thereafter and did not return to normal for 3 months after the ictus. Our own studies have demonstrated alterations typical of an acute phase response with high levels of plasma fibrinogen and low albumin/globulin ratios immediately following stroke. Finally, evidence of platelet activation has been observed during acute stroke. This could also reflect ongoing thrombin generation. The generation of thrombin and other activated clotting factors such as factor Xa is now known to be governed not only by events such as tissue injury, but also by the ability of the “naturally occurring” anticoagulants to inhibit their prethrombotic activity.

Inhibitors of Thrombosis

The schema outlined in the lower portion of Figure 1 summarizes the principal components and actions of some of the inhibitors of coagulation. Both vascular endothelial cells and plasma proteins are key components of this system. The anticoagulant factors include antithrombin III (ATIII), protein C, and protein S. The fibrinolytic system, which includes tissue plasminogen activator (t-PA), plasminogen, and plasmin, serves to limit thrombosis. ATIII is synthesized by the liver and binds to the heparin-like substance, heparan, which is expressed on the surface of endothelial cells and markedly amplifies the ability of ATIII to inactivate thrombin and other activated clotting factors.

The conversion of fibrinogen to fibrin is inhibited when thrombin binds to an endothelial cell surface protein, thrombomodulin. As illustrated, thrombin binding to thrombomodulin promotes the activation of protein C, an anticoagulant protease that inactivates thrombin activated factors V (Va) and VIII (Villa), and may also promote fibrinolysis. Protein S, which exists both as a free form and bound to a complement binding protein, accelerates protein C inactivation of Va and Villa. Only free protein S is active as an anticoagulant. Deficiencies or functional abnormalities of ATIII, protein C, or free protein S or alterations in the regulating systems controlling the function of these proteins may predispose to thrombosis.
ATIII, Protein C, and Protein S: Hereditary and Acquired Deficiencies

Hereditary deficiencies of ATIII, protein C, and protein S are recognized by low (e.g., 50%) concentrations of the protein or by low functional activity in laboratory assays. This distinction is important since assays that determine the concentration of the protein may not identify mutations producing molecules with abnormal function. Approximately 20% of patients with recurrent venous thrombosis will be found to have deficiencies or defects of these proteins. The great majority of patients with prethrombotic states will experience deep or superficial venous thromboses. However, CNS thrombotic events have also been described, but from the few cases that have been reported, cerebral venous thrombosis appears to occur more often than arterial stroke or TIA. Even lesser deficiencies of ATIII, for example, 70–75% of normal, may suffice to produce a prethrombotic state. The risks of thrombosis in persons with hereditary deficiencies of ATIII, protein C, or protein S may increase as a consequence of aging, use of oral contraceptives or pregnancy, tobacco abuse, or following trauma or surgery.

Acquired deficiencies of ATIII and proteins C and S may also be associated with prethrombotic states and with brain infarction. Although controlled prospective studies have not been performed, when stroke is associated with acquired abnormalities of these proteins, other major diseases or special circumstances are often present. For example, alterations in either the concentration or function of the anticoagulant factors may occur in pregnancy, in women taking oral contraceptives, and in patients with malignancies, hepatic failure, or the nephrotic syndrome. Acute fluctuations of anticoagulant protein levels can also follow plasmapheresis and hemodialysis. Paradoxical thromboembolism to brain in young persons with unrecognized atrial septal defects may be due to underlying venous thromboembolism. Thus, patients with the above conditions who experience TIA, stroke, or amaurosis fugax should be evaluated for a prethrombotic state.

The anticoagulant proteins may be reduced in liver disease, but concurrent reduction in procoagulant factors usually predisposes to bleeding rather than thrombosis. However, a postsurgical prethrombotic state has been described in children following liver transplantation. These children are found to have reductions in ATIII and protein C, with an increase in an inhibitor to t-PA. A similar prethrombotic state might occur in adult patients with perioperative stroke.

Although hereditary and acquired deficiencies of ATIII and proteins C and S have been associated with brain infarction, the contributions of the anticoagulant proteins and their regulation to the pathogenesis of stroke remain to be clarified. Two studies, one retrospective, have failed to document deficiencies in the natural anticoagulant proteins in patients who experience stroke. However, we have studied several patients with recurrent brain infarctions who had persistent deficiencies of free protein S as their only risk factor for thrombosis, and a recent prospective study found free protein S deficiency in 23% of patients with stroke of uncertain cause. All but one patient in this series were under age 55. In another prospective study D’Angelo and colleagues reported that a reduced protein C level measured at the time of acute stroke was correlated with a poor outcome, but no differences in ATIII levels were found between survivors and nonsurvivors of stroke. There has not been a prospective investigation of the relationship between acquired long-term deficiencies of these proteins and the occurrence of stroke.

Antiphospholipid Antibody Syndrome

The definition and spectrum of the immune mediated thrombotic disorder, APA syndrome, have
been detailed in several excellent review papers.²⁰⁻²² Diagnostic criteria include venous or arterial thrombotic events (including stroke, retinal artery occlusion, or TIAs), recurrent fetal loss, or thrombocytopenia associated with antcardiolipin antibodies (ACLA) or the lupus anticoagulant.²³,²⁴ APA bind to negatively charged phospholipids, including cardiolipin, phosphatidylyserine, phosphatidylinsitol, and phosphatidyethanolamine. Included in the spectrum of clinically important APA are antcardiolipin antibodies, which are the antibodies responsible for a false positive VDRL test, and the lupus anticoagulant. Patients with the APA syndrome may have a single immunoglobulin class (e.g., IgG) or several classes of antibodies in their serum. Many patients with the lupus anticoagulant also have ACLA but not vice versa, and evidence now indicates that the lupus anticoagulant and ACLA are probably unique antibodies.

The neurologic events that have been associated with APA include brain infarction, TIA, amaurosis fugax, migraine-like headaches, and acute ischemic encephalopathy.²⁵⁻²⁷ Significantly, the APA syndrome appears to be related to recurrent stroke and to multi-infarction dementia.²⁷ Often, dementia is the presenting feature, and a history of previous stroke or like episodes is not obtained. In patients with recurrent stroke, cortical and subcortical wedge-shaped infarctions and, rarely, lacunar stroke are found on CT scan. Since multiple arteriolar occlusions with platelet fibrin thrombi are found at post-mortem examination, it is surprising that lacunar strokes are not more common. The APA syndrome may present at any age and be associated with typical stroke risk factors, but the vascular occlusive symptoms usually appear by the fifth decade of life. Younger patients with APA often report a history of migraine-like headaches which have atypical features. In particular, the APA syndrome should be suspected in patients with atypical migraine and so-called “bright objects” on the MRI scan. Our experience suggests that the bulk of neurologic events in the APA syndrome are usually related to vascular ischemia in the CNS. In addition to in situ thrombosis, endocardial or cardiac valvular lesions may be a source of thromboemboli. Finally, APA are sometimes detected in patients with AIDS, Guillain-Barré syndrome, and myelopathy, which suggests that APA may also be involved in immune mediated nonvascular neurologic injury.

Although the APA syndrome often presents with isolated nervous system symptomatology, other manifestations may also occur. For example, a bland thrombotic vasculopathy may involve the vessels of multiple organ systems, especially skin, heart, and lungs. The presentation is often initially suggestive of systemic vasculitis. When the disease involves the skin (e.g., livedo reticularis), a skin biopsy in which fibrin-platelet thrombi are identified without a perivascular infiltrate of inflammatory cells may help establish the diagnosis.²⁶ In individuals with APA, the prevalence of associated rheumatic diseases, especially lupus erythematosus, is high, but over 50% of persons with APA will not have obvious rheumatologic disease. A genetic predisposition to the development of these antibodies may exist since familial occurrence of the APA syndrome has been reported.²⁸ The term “primary APA syndrome” has been proposed for those patients without associated rheumatic disease.²²

Although multiple mechanisms of thrombotic action have been proposed, no underlying explanations for the association of APA with a predisposition to thrombosis have emerged.²⁴ APA in some instances appear to inhibit prostacyclin (PGI₂) production by vascular endothelium. Other studies have suggested that these antibodies may interfere with the function of the natural anticoagulant proteins or with fibrinolysis or that they may interact with platelet membranes to produce platelet activation.²⁹,³⁰

The relationship between the APA syndrome and circulating levels of the anticoagulant proteins appears weak. Plasma protein S deficiency has been reported in some but not all patients with the lupus anticoagulant. As yet, there are no reports of inhibition of protein S activity by APA in vitro.²⁹ Reduction in the plasma levels of the anticoagulant proteins was rarely found in a large number of stroke patients from our institution with the APA syndrome. However, several lines of evidence indicate that APA may occasionally interfere with the function of protein C. Using cultured endothelial cells, Cariou and colleagues³⁰ demonstrated that the lupus anticoagulant from several patients interfered with the ability of the thrombin/thrombomodulin complex to activate protein C. Recently, a fatal thrombotic disorder was reported in a patient who had an IgG paraprotein which inhibited the functional activity of protein C.³¹ Whether the paraprotein had antiphospholipid specificity was not determined although protein C and protein S levels were normal and the immunoglobulin failed to recognize either purified protein C or activated protein C. These studies suggest that APA and possibly other immunoglobulins could interfere with the thrombin/thrombomodulin activation of protein C. In another instance a lupus anticoagulant was found that blocked PGI₂ synthesis but failed to inhibit either protein C activation or thrombin binding to thrombomodulin. To date, the total number of lupus anticoagulants or APA that have been shown to inhibit protein C activation have been limited, and the antigenic specificity of the immunoglobulin responsible for the inhibition has not been determined in most cases.

One reported patient with APA developed a deficiency of functional ATIII. The APA could possibly cross-react with endothelial cell bound heparan,³² leading to decreased binding or function of ATIII, although evidence for this hypothesis is not yet available.

The fibrinolytic process is also a potential target of APA action.²⁸ Intrinsic fibrinolysis requires interac-
tions of the contact coagulation factors, prekallikrein, factor XII, and high molecular weight kininogen (HMWK), to convert plasminogen to plasmin. A major inhibitor of this system is C1 esterase inhibitor. The inhibition of prekallikrein demonstrated in several patients with APA could have been due to the presence of supranormal levels of C1 esterase inhibitor. APA could possibly provoke increased synthesis or release of C1 esterase inhibitor from vascular endothelial cells. Other reports suggest that patients with APA may have decreased vascular release of t-PA, disrupting the extrinsic fibrinolytic mechanisms of the vasculature.24

Finally, activated protein C may have a profibrinolytic role via inhibition of plasminogen activator inhibitor, the fast-acting inhibitor of t-PA. Increased plasminogen activator inhibitor levels have been reported to decrease t-PA activity and have been associated with venous thrombosis. Therefore, APA may not only inhibit the ability of activated protein C to neutralize factors Va and VIIIa but also the ability to promote fibrinolysis. Recently, a preliminary report suggested that 17 of 24 patients with thrombosis and elevated titers of ACLA had elevated plasminogen activator inhibitor levels.33

Heparin-Associated Thrombocytopenia
Although relatively uncommon, the heparin-associated thrombocytopenia syndrome has provided insight into an important mechanism of immune mediated thrombosis. Individuals developing this syndrome are at great risk for major arterial thrombotic events, including stroke, with a high mortality rate.34-35 Atkinson et al36 have emphasized a relationship between heparin-associated thrombocytopenia and ischemic stroke following carotid endarterectomy. In a typical patient moderate to severe thrombocytopenia develops 5-10 days after heparin has been started. Although platelet counts may be markedly reduced, thrombotic rather than hemorrhagic complications are usually encountered. Cines and colleagues32 studied 27 patients with the heparin-associated thrombocytopenia syndrome and suggested that an IgG antibody from the plasma of these subjects bound to the heparan on the surface of endothelial cells in culture and stimulated the synthesis and expression of the procoagulant tissue factor on the endothelial cell surface.

It is possible that APA or other antibodies might also promote endothelial cell or monocye tissue factor expression in vivo. However, in preliminary studies we were unable to elicit tissue factor expression on the surface of human umbilical vein endothelial cells in culture when incubated with sera from patients with high titer ACLA (unpublished data).

Laboratory Evaluation of Prethrombotic States in Stroke
Most diagnostic studies for the prethrombotic states, such as the lupus anticoagulant screen, ACLA titers, and anticoagulant proteins, are first performed during or immediately after acute stroke. However, many of the assays (e.g., lupus anticoagulant, protein C or S, or ATIII) may be influenced by acute illness or therapeutic anticoagulants. Consequently, the determinations should be repeated during convalescence when the patient is not receiving oral anticoagulants. Often, patients who have a lupus anticoagulant will have ACLA as well. Nevertheless, since these antibodies appear to be distinct, both a lupus anticoagulant screen and ACLA assays may be required to detect antiphospholipid antibodies in the blood. We favor the kaolin clotting time for a sensitive examination of lupus anticoagulant while others prefer a PTT test that uses a sensitive phospholipid reagent or the dilute Russel viper venom time. If a patient is receiving warfarin, the assays of the anticoagulant proteins should be performed after warfarin has been withheld for 2-3 weeks. Currently, assays that measure the functional activity of ATIII and protein C are widely used. In contrast, assays for free protein S usually measure protein S antigen rather than function although, hopefully, simple and reliable activity assays will soon be available.

Treatment
Unfortunately, treatment of prethrombotic states in patients with stroke remains largely empiric, and well-designed prospective clinical trials are critically needed. Patients with protein C or S or ATIII deficiency who have experienced prior thrombotic events usually require long-term anticoagulation with warfarin. However, it should be appreciated that in patients with protein C or protein S deficiency, the administration of warfarin without antecedent heparin may result in warfarin-induced skin necrosis due to microvascular thrombosis.10

A variety of treatments for patients with APA and thrombosis have been recommended, but their efficacy remains unknown. For patients with minor symptoms or for those who are asymptomatic, low dose aspirin or other antiplatelet agents have been recommended. In patients who have experienced stroke or other major thrombotic events, warfarin may be tried, especially in those with very high titers of APA or in patients who have not responded to antiplatelet therapy. Additional modalities that may be useful include immunosuppressive therapy with either prednisone or a combination of prednisone and cyclophosphamide. Patients who may benefit include those who have suffered multiple, acute or life-threatening thromboses, such as severe ischemic encephalopathy, or who have other indications for immunosuppressive therapy such as active lupus erythematosus. Plasmapheresis may lower the titer of APA during the acute phase of an illness in selected patients although proof of benefit is not yet clear.36

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B M Coull and S H Goodnight

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