The annexins are a family of highly homologous phospholipid binding proteins, which share a four-domain structure, with one member of the family – annexin VI – having a duplication consisting of eight domains. Thus far, ten annexins have been described in mammals. Although the biological functions of the annexins have not been definitively established, two human diseases involving annexin abnormalities (‘annexinopathies’) have been identified as of the time of writing. Overexpression of annexin II occurs in the leukocytes of a subset of patients having a hemorrhagic form of acute promyelocytic leukemia. Underexpression of annexin V occurs on placental trophoblasts in the antiphospholipid syndrome and in preeclampsia. Also, an animal model has been described in which annexin VII is underexpressed and is associated with disease, but the relevance of this animal model to human disease is not yet understood. Future research is likely to elucidate additional ‘annexinopathies’. © 2000 Elsevier Science B.V. All rights reserved.

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1. Overview of annexins

The annexins (for extensive review see [1]) are a family of phospholipid binding proteins that were originally isolated by various investigators from various tissues and assigned their previous names based upon their tissue of origin or on their presumed functions. With amino acid and nucleotide sequencing came the recognition of identities among several independently isolated annexins, their structural relationships and differences, and their shared properties of binding calcium and phospholipids. Thus far, several hundred different annexins, including ten in mammals (I–VIII, XI and XIII), have been described. The remainder have been described in Droso phila, Hydra, protozoa and a wide range of plants [2]. Their ‘canonical structure’ is composed of repetitive homologous domains consisting of sequences of about 70 amino acids, with most of the annexins having four of these domains. The amino-terminal tails of the various annexins are unique and are believed to confer the functional specificities.

While their structural homology and the shared attributes of binding to calcium and to phospholipid suggest the possibility of common functional themes, these have remained elusive. Roles in physiology and human disease have recently been proposed for some of these proteins.
2. Annexin II and acute promyelocytic leukemia

Annexin II has been proposed to play a role in the process of fibrin clot dissolution. The protein promotes fibrinolysis on the apical surfaces of vascular endothelial cells by serving as a platform for the binding of plasminogen and tissue plasminogen activator [3]. This permits the efficient cleavage of plasminogen by tissue plasminogen activator to produce the fibrinolytic enzyme, plasmin.

It has been known for some time that subsets of patients with acute promyelocytic leukemia are prone to develop a severe bleeding disorder [4]. Among the hallmarks of this disorder are hypofibrinogenemia and excessive fibrinogenolysis for which the pathophysiology has not been previously understood. This condition had been previously attributed to disseminated intravascular coagulation [4]. Recently, overexpression of annexin II has been described in one such subset of patients with acute promyelocytic leukemia [5]. Annexin II is abnormally elevated in patients having the 15:17 translocation form of acute promyelocytic leukemia and is associated with excessive fibrinolysis. This proposed mechanism (illustrated in Fig. 1) is supported by the finding that leukemia cell-mediated fibrinolysis was inhibited with polyclonal anti-annexin II antibodies. Also, fibrinolysis could be increased in a non-fibrinolytic acute promyelocytic leukemia cell line by inducing the expression of annexin II [5]. At this point, no other diseases of annexin II overexpression have yet been identified. There have not yet been described any clinical annexin II deficiency states.

3. Annexin V and the antiphospholipid syndrome

The antiphospholipid (aPL) syndrome is an autoimmune condition that occurs in patients having antibodies against anionic phospholipid-protein com-

![Fig. 1. Proposed mechanism of hemorrhage in acute promyelocytic leukemia. Plasmin is formed on assembly of plasminogen and tissue plasminogen activator (t-PA) on cell surface-associated annexin II (A). At the cell surface, plasmin is protected from its primary inhibitor, α2-plasmin inhibitor (α2-PI), which is produced in the liver. Once released, plasmin rapidly forms an irreversible, inactive complex with α2-PI. Plasmin is generated on the surface of endothelial cells and, to a lesser extent, on other cells. In leukemias other than acute promyelocytic leukemia, released plasmin is neutralized by α2-PI, and the plasmin-α2-PI complexes are cleared in the liver. In acute promyelocytic leukemia, plasmin is generated at an abnormally high rate because of overexpression of annexin II on the leukemic cells (B). As a result, α2-PI is consumed, and active plasmin accumulates in the plasma. The unopposed fibrinolytic activity of plasmin causes a hemorrhagic disorder. (Reprinted with permission from The New England Journal of Medicine [4].)
plexes. The syndrome manifests as vascular thromboembolism or recurrent pregnancy losses. For recent comprehensive reviews the reader is referred to [6,7]. The disorder is classified as ‘primary’ in the absence of another autoimmune condition, such as systemic lupus erythematosus, and ‘secondary’ in the presence of such disorders.

aPL antibodies are identified by their reactivity to anionic phospholipids (or protein-phospholipid complexes) in solid phase immunoassays and by their inhibition of phospholipid-dependent coagulation reactions (the ‘lupus anticoagulant’ effect). The pathophysiologic mechanism(s) of this syndrome have been confusing because of the apparent multiplicity of antigenic determinants recognized by the antibodies and the uncertainty about whether any of these specificities are causally relevant to the disease process. While a large number of effects have been described for the antibodies in vitro and in cell culture systems [6], aPL antibody-mediated disruption of annexin V binding to phospholipid offers a particularly intriguing mechanism.

Annexin V has potent anticoagulant properties in vitro and significantly prolongs the phospholipid-de-
dependent coagulation reactions. The anticoagulant effect is due to the ability of this protein to displace coagulation proteins from phospholipid surfaces [8]. Interestingly, there is evidence that annexin V forms clusters on exposed phospholipid [8] in the form of two-dimensional crystalline arrays [9,10]. This shield of annexin V on the phospholipid surface blocks these phospholipids from availability for any coagulation reactions and also reduces the lateral mobility of any reactants that are already bound to the phospholipid membrane.

Annexin V is necessary for the maintenance of placental integrity [11]; infusion of pregnant mice with polyclonal anti-annexin V antibodies resulted in placental infarction and pregnancy wastage. The protein is expressed in an apparently constitutive manner by human placental trophoblasts and has been immunolocalized to the apical surfaces of the syncytiotrophoblasts lining the placental villi [12], where it is densely expressed. Here, the protein is present at the interface between the maternal blood which circulates through the intervillous space and the fetal trophoblasts. Deficiency of annexin V has been described in placentas of patients with the antiphospholipid syndrome [13].

Recently, it was reported that annexin V expression is decreased on trophoblasts of preeclamptic placentas and that the degree of the decrease correlated with elevation of markers for activation of blood coagulation [14]. Also, annexin V is reduced on placental villi [15] and on cultured placental trophoblasts exposed to IgG fractions from aPL patients [16], and by a monoclonal antiphosphatidylserine antibody [17]. This antibody-mediated reduction of annexin V is associated with acceleration of plasma coagulation [16,18] and increased binding of prothrombin [17], and can occur on non-cellular phospholipid surfaces [18,19]. The mechanism for aPL antibody-mediated acceleration of coagulation is illustrated in Fig. 2.

Taken together, the available data support the hypothesis that annexin V has a thrombomodulatory function on the surfaces which line the placental and systemic vasculatures. Reduction of annexin V in preeclampsia and in the aPL syndrome is associated with pregnancy complications and losses.

4. Annexin VII knockout mice

The annexin VII gene has been knocked out and the nullizygous annexin VII (−/−) phenotype has been found to be lethal at embryonic day 10 because of cerebral hemorrhage [20]. The heterozygous annexin VII (+/−) mouse, although expressing only low levels of annexin VII protein, is viable. That phenotype is associated with a substantial defect in insulin secretion, although the insulin content of the islets is 8–10-fold higher in the mutants than in the normal littermate control. This appears to be due to a change in the ability of inositol 1,4,5-trisphosphate (IP(3))-generating agonists to release intracellular calcium. The major molecular consequence of lower annexin VII expression appears to be the profound reduction in IP(3) receptor expression and function in pancreatic islets [20]. The relevance of these findings to human disease has not yet been established.

5. Summary and conclusions

We now have significant evidence that two of the annexins, II and V, play roles in the physiologic control of blood coagulation reactions and also that abnormalities of the expression of these proteins are associated with clinical disease. Annexin II is overexpressed on leukocytes in patients with acute promyelocytic leukemia having the 15:17 translocation, which correlates with increased fibrinolysis and bleeding. Annexin V is decreased on placental trophoblasts and on endothelial cells in the antiphospholipid syndrome, an antibody-mediated effect which is associated with pregnancy losses and with thrombosis. In animal models, knockout of another annexin has been associated with disease. Deficiency of annexin VII has been associated with decreased insulin release and alterations in the morphology of pancreatic islets. These abnormalities have not yet been related to human diseases.

We may anticipate that forthcoming research will reveal additional ‘annexinopathies’ which will include a broad range of genetic and acquired abnormalities of the human annexins.
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