The Role of Complement in the Antiphospholipid Syndrome: A Novel Mechanism for Pregnancy Morbidity

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Objectives: Despite the experimental research data on antiphospholipid syndrome (APS), the pathogenesis of thrombosis and fetal loss remains unknown. The objective of this study was to analyze the major advances in the field of complement activation as a possible thrombosis mechanism in the APS.

Methods: The authors conducted a systemic analysis of the English literature and summarized both animal and human data that indicate the inappropriate complement activation as a mechanism causing thrombosis in the APS.

Results: The important role of complement activation in the pathogenesis of fetal loss was established using mice deficient in a complement regulatory protein. Further studies have shown that the infusion of human IgG antiphospholipid antibodies (aPL) induced fetal loss in pregnant mice, an effect that was abrogated by the concurrent administration of a C3 convertase inhibitor. Further studies suggested that C5a and neutrophils were the key components responsible for fetal injury. Moreover, use of F(ab)′2 fragments of aPL suggested the complement activation occurred mainly via the classical pathway. Other studies using models of induced thrombosis suggested that antibodies against β2GPI required the presence of terminal complement components to induce thrombus formation, and mice deficient in C3 or C5 were found to be resistant to aPL-induced thrombosis. Based on the aforementioned findings, it has been suggested that heparin prevents fetal loss in patients with APS by inhibiting complement activation rather than by its anticoagulant effect.

Conclusions: The studies on complement are significant because they shift the focus of research in APS from thrombosis to inflammation. However, as human data are limited, more clinical research is necessary before the above findings translate in changes in the management of APS.

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The antiphospholipid syndrome (APS) is defined as the combination of antiphospholipid antibodies (aPL) with either thrombosis (arterial or venous) or pregnancy morbidity. According to a recent international consensus statement, pregnancy morbidity is defined as at least 3 otherwise unexplained embryonic losses (ie, before the 10th week of gestation) or at least 1 otherwise unexplained fetal loss (ie, after the 10th week of gestation) or at least 1 premature birth before the 34th week of gestation because of eclampsia or severe preeclampsia or placental insufficiency (1). Pregnancy morbidity is among the more frequent clinical presentations of the primary APS and in turn APS syndrome is a major cause of pregnancy morbidity (2).

Although the pathogenetic potential of aPL has been established and their antigenic targets largely defined, there is still uncertainty regarding the exact pathogenetic mechanisms in APS. Despite this uncertainty, it is generally accepted that the basic mechanism underlying all clinical manifestations of APS is thrombosis (3). To ex-

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plain thrombosis in APS, almost all elements involved in thrombus formation have been investigated. Thus in patients with APS, abnormalities in the clotting cascade, abnormal endothelial cell and platelet activation, and abnormalities in fibrinolysis (4-6) have been found. Until recently, inappropriate complement activation had not been investigated as a possible mechanism causing thrombosis in the APS, although it is known that complement activation activates endothelial cells and platelets, which thus acquire a procoagulant phenotype. However, recent findings from experimental animals, suggesting that complement activation is involved in pregnancy loss (7), led to the investigation of this mechanism in the pathogenesis of APS-related pregnancy morbidity.

The seminal study that suggested an important role for complement activation in fetomaternal tolerance was the 1 by Xu and coworkers in 2000 (7). This group investigated the role of Crry, a complement protein that regulates the deposition of C3 and C4 in rodents; its function is similar to the human complement regulatory proteins decay accelerating factor (DAF) and membrane cofactor protein (MCP). The investigators found that complete Crry deficiency resulted in embryonic developmental arrest and embryonic lethality due to complement deposition and placental inflammation. Interestingly, breeding of $C^{-/-}$ mice rescued $Crry^{-/-}$ mice from lethality, suggesting that deaths in $Crry^{-/-}$ embryos required the presence of C3. This fact in combination with the presence of cell-surface C3 strongly suggested that embryonic deaths in $Crry^{-/-}$ were caused by uncontrolled complement activation (7). It is noted however that the authors suggested that fetal death was due to the inflammatory response caused by complement activation rather than thrombosis.

Based on the above data, Holers and coworkers, to investigate the role of complement in APS, used a mouse model in which passive transfer of human IgG containing aPL (aPL-Ig) induced fetal loss (8). The investigators found that inhibition of the complement cascade in vivo prevents fetal loss and growth retardation, whereas C3 null mice were resistant to fetal injury induced by aPL antibodies (8). Thus, the authors showed that complement activation is a necessary step in the pathogenesis of APS-associated pregnancy morbidity. However, they could not locate the site of the abnormal activation because in their model both the classical and the alternative pathways were inhibited. Trying to extend their findings in APS-associated thrombosis, they studied the dynamics of thrombus formation using a model of in vivo microcirculation thrombus formation monitoring, which had been used by Pierangeli and coworkers (9) and found that blocking of C3 activation decreased significantly the size of aPL-induced thrombosis. Based on these data, Holers and coworkers proposed a 2-hit hypothesis: aPL antibodies target the placenta specifically and cause platelet and endothelial cell activation, inducing a procoagulant state. However, this is not sufficient to cause fetal loss or growth retardation. Complement activation increases the production of mediators such as C3a, C5a, and C5b-9 MAC, which promote further platelet and endothelial cell activation, leading to inflammation, tissue damage, and, finally, fetal loss (8).

The implication of the classical pathway and more specifically of the C5 in APL-associated pregnancy complications was shown by Girardi and coworkers. The investigators showed that aPL-induced pregnancy loss required the Fc fragment of immunoglobulin but it was not mediated through FcγR, which is a pattern of requirements compatible with complement activation via the classical pathway. Experiments with animals genetically deficient in C4 and C5, as well as administration of monoclonal antibodies against C5, suggested that pregnancy morbidity was mediated via the classical pathway (10). However, the same investigators found that the alternative pathway contributes to the pathogenesis of APS-mediated fetal morbidity as well (10,11). Furthermore, Berman and coworkers demonstrated that following aPL administration there was a C5-dependent increase in decidual and systemic TNF-α levels, concluding that TNF-α acts as a downstream effector of C5 activation and therefore suggesting TNF blockade as a potential therapy for APS-related pregnancy morbidity (12).

Tissue factor (TF) has been reported as a contributor of both inflammation and coagulation in APS. Ritis and coworkers showed in vitro that aPL-induced complement activation led to signaling via the C5a receptor on neutrophils, TF induction on neutrophils, and, finally, increased procoagulant activity (13). Redecha and coworkers using mouse models confirmed both the importance of TF expression on neutrophils in the pathogenesis of aPL-induced fetal loss and the functional linkage among C5a, neutrophil activation, and fetal injury (14). Actuated by the data demonstrating that TF participates in both coagulation and inflammatory processes through its interaction with protease activated receptors (PARs) (15), the same investigators attempted to elucidate the possible role of TF/PAR2 interaction in the inflammation-driven trophoblast injury (16). What they demonstrate was that the genetic reduction of the TF signaling through PAR2 as well as the breeding of $Par2^{-/-}$ mice treated with aPL exhibited reduced neutrophil activation and normal pregnancies. Notably, simvastatin and pravastatin were found to decrease TF and PAR2 on neutrophils and protect from aPL-mediated fetal death (16).

As a consequence of the aforementioned observations and of the well-known anticomplement activity of heparin (17), the therapeutic mechanism of anticoagulant treatment in APS was reassessed. Earlier research by Di Simone and coworkers using cultured trophoblast cells and inhibition immnoassays suggested that low molecular weight heparin inhibited binding of aPL to trophoblast cells, thus "protecting" the phospholipids of trophoblasts (18). Girardi and coworkers showed in vitro and in vivo that both unfractionated heparin and low molecular weight heparin—but not other anticoagulants such as hirudin and fondaparinux—inhib-