Complement C5a receptors and neutrophils mediate fetal injury in the antiphospholipid syndrome

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Antiphospholipid syndrome (APS) is defined by recurrent pregnancy loss and thrombosis in the presence of antiphospholipid (aPL) Ab’s. Currently, therapy for pregnant women with APS is focused on preventing thrombosis, but anticoagulation is only partially successful in averting miscarriage. We hypothesized that complement activation is a central mechanism of pregnancy loss in APS and tested this in a model in which pregnant mice receive human IgG containing aPL Ab’s. Here we identify complement component C5 (and particularly its cleavage product C5a) and neutrophils as key mediators of fetal injury, and we show that Ab’s or peptides that block C5a–C5a receptor interactions prevent pregnancy complications. The fact that F(ab)’2 fragments of aPL Ab’s do not mediate fetal injury and that C4-deficient mice are protected from fetal injury suggests that activation of the complement cascade is initiated via the classical pathway. Studies in factor B–deficient mice, however, indicate that alternative pathway activation is required and amplifies complement activation. In contrast, activating FcyRs do not play an important role in mediating aPL Ab–induced fetal injury. Our findings identify the key innate immune effectors engaged by pathogenic autoantibodies that mediate poor pregnancy outcomes in […]

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within the placenta. We investigated this mechanism because it is well established that activated complement fragments themselves have the capacity to bind and activate inflammatory and endothelial cells as well as to induce a prothrombotic phenotype (15, 16). The validity of this hypothesis was demonstrated in our recent studies showing that in the murine model of APS, blockade of C3 activation prevents fetal loss and growth restriction induced by passive aPL Ab transfer (17). Nevertheless, the effects of tissue injury, the role of individual complement activation pathways, and the precise targets for treatment have remained unknown.

In addition to causing complement activation, aPL Ab's may induce injury through inflammatory pathways involving activating Fcγ receptors (FcγRs) and neutrophils. These mediators could also link production of pathologic IgG to development of overt clinical disease. In the current work we examined these three mechanisms to determine their relative importance in aPL Ab–mediated fetal loss. The results of these studies provide a conceptual framework within which rational therapeutic strategies and interventions can be developed.

Methods

Mice. Adult mice (2–3 months old) were used in all experiments. BALB/c mice were purchased from Taconic Farms (Germantown, New York, USA). FeRγ−/− mice backcrossed to BALB/c mice were provided by Jeffrey Ravetch (Rockefeller University, New York, New York, USA) (18). C4−/− mice were generated by homologous recombination and backcrossed to C57BL/6 for 17 generations (19, 20). C5−/− (B10.D2-H2−H2-T18− Hc/o/Sn) and the C5−/− background-strain mice (B10.D2-H2−H2-T18− Hc/o/SnJ) were obtained from The Jackson Laboratories (Bar Harbor, Maine, USA). C5a receptor–deficient (C5aR−/−) mice were generated by targeted deletion of the murine C5aR gene and determined to be completely C5aR deficient by PCR, Northern blot, and immunohistochemistry analyses (T.J. Hollman and R.A. Wetsel, data not shown). C5aR−/− deficient animals were backcrossed with C57BL/6 mice. Heterozygous C5aR+/− backcrossed mice were interbred, and the resulting C5aR+/+ and C5aR−/− litters were used for studies. Mice deficient in factor B (fB) were generated by targeted deletion in bone marrow correlates with granulocyte maturation, and in peripheral blood, rat anti-mouse granulocyte RB6-8C5 mAb (PharMingen, San Diego, California, USA) (100 μg, intraperitoneally) that reacts with Ly6G (Gr-1 myeloid differentiation antigen); an IgG2b mAb was the isotype control. The level of Ly6G antigen expression in bone marrow correlates with granulocyte maturation, and in peripheral blood, rat anti-mouse granulocyte RB6-8C5 mAb recognizes neutrophils and eosinophils (28–30). Neutrophil depletion was observed 24 hours after administration of anti-mouse granulocyte mAb (anti-Gr) and persisted through day 15. Mice were sacrificed on day 15 of pregnancy, uteri were dissected, fetuses and placentas were weighed, and fetal resorption rates were calculated (number of resorptions per total number of formed fetuses and resorptions). Resorption sites are easily identified and result from loss of a previously viable fetus. Functional C3 activity in serum was measured using the previously described zymosan assay (31).

Immunohistochemistry. Decidua were removed from mice on day 8 of pregnancy, 60 minutes after administration of aPL-IgG, frozen in OCT compound, and cut into 10-μm sections. After quenching endogenous peroxidase with 1% H2O2 in methanol and blocking non-
FcR band was identified by comparing serum incubations. Deciduas, including embryos, from day 15 of pregnancy were sacrificed on day 15 of pregnancy, fetuses were weighed, and frequency of fetal resorption (Figure 1a). In surviving fetuses from FcRγ−/− mice there was a 36% decrease in weight. (b–e) Immunohistochemical analysis of decidual tissue from day 8 of pregnancy. Sections were stained with goat anti-human IgG, the chromogen was DAB (brown), and the counterstain was hematoxylin. Human IgG was deposited in deciduas from healthy non-autoimmune individual (NH-IgG), three different patients with APS (aPL-IgG1, aPL-IgG2, aPL-IgG3), F(ab)′2 fragments from a pool of aPL-IgG from patients 2 and 3 [aPL-F(ab)′2], or human monoclonal aPL Ab (aPL mAb) on days 8 and 12 of pregnancy. Mice were sacrificed on day 15 of pregnancy, fetuses were weighed, and frequency of fetal resorption calculated (n = 4–7 mice/group). (a) Treatment with all intact aPL-IgG preparations and aPL mAb caused an increase in fetal resorptions in FcRγ+/+ mice ( Figure 1). Administration of aPL-F(ab)′2 did not affect pregnancy outcome. FcRγ−/− mice were not protected from fetal loss induced by intact aPL-IgG. *P < 0.05 versus NH-IgG, Student’s t test. Administration of aPL-F(ab)′2 did not affect pregnancy outcome. FcRγ−/− mice were not protected from fetal loss induced by intact aPL-IgG. *P < 0.05 versus NH-IgG, Student’s t test. In surviving fetuses from FcRγ−/− mice there was a 36% decrease in weight. (b–e) Immunohistochemical analysis of decidual tissue from day 8 of pregnancy. Sections were stained with goat anti-human IgG, the chromogen was DAB (brown), and the counterstain was hematoxylin. Human IgG was deposited in deciduas from FcRγ−/− mice within 60 minutes of administration of aPL-IgG (b) or aPL-F(ab)′2 (c), whereas no IgG was detected in deciduas from FcRγ−/− mice treated with NH-IgG (d). Deposition of human IgG was similar after treatment with aPL-IgG in FcRγ−/− (e) and FcRγ+/+ mice (b). Data are representative of observations from three to six decidua from mice in each experimental group. Original magnification was ×200.

**Results**

Activating FcγRs are not required for aPL Ab–induced pregnancy complications. The Fc domain of pathogenic IgG may initiate tissue damage by binding FcγR on effector cells and/or initiating activation of complement. As a first approach to determine the role of FcγR in pregnancy loss induced by aPL Ab’s we compared the consequences of treating pregnant mice with polyclonal IgG isolated from APS patients and F(ab)′2 fragments prepared from the same IgG source. Passive transfer of IgG from three different patients with high-titer aPL Ab’s (aPL-IgG) (>140 GPL units) consistently caused a fourfold increase in the frequency of fetal resorption (Figure 1a). In contrast, treatment with F(ab)′2 fragments of aPL-containing IgG did not
affect the frequency of fetal loss (Figure 1a). Fetal loss in mice treated with F(ab)′_2 fragments of aPL-IgG was similar to that observed in mice treated with NH-IgG (Figure 1a). In addition, growth restriction induced by treating pregnant mice with intact aPL-IgG was also absent in surviving fetuses of mice treated with aPL-IgG F(ab)′_2 (average fetal weight: aPL-IgG, 213 ± 42 mg; aPL-IgG F(ab)′_2, 343 ± 43 mg; NH-IgG, 326 ± 32 mg; aPL-IgG F(ab)′_2 versus aPL-IgG, P < 0.05). Importantly, deposition of human F(ab)′_2 IgG in decidual tissues was similar in mice treated with aPL-IgG and aPL-IgG F(ab)′_2 (Figures 1, b and c), and no human IgG deposition was observed in deciduas from mice treated with NH-IgG (Figure 1d).

Given our finding that the Fc portion of IgG is necessary for aPL Ab–mediated injury, we considered the possibility that aPL Ab's deposited in the decidua initiate inflammation, thrombosis, and fetal demise by cross-linking stimulatory FcγRs expressed on monocytes, neutrophils, platelets, or mast cells. To examine the role of the FcγR in aPL Ab–induced pregnancy loss, we studied mice with targeted deletion of the common γ subunit (FcγR–/–) that is required for signaling by activating FcγRs, high-affinity FcγRI, and low-affinity FcγRIII (18). Although FcγR–deficient mice are reported to have less-severe or undetectable Ab-dependent experimental hemolytic anemia, thrombocytopenia, and glomerulonephritis (32), we found that FcγR–/– mice were not protected from poor pregnancy outcomes after passive transfer of aPL-IgG (Figure 1a). To exclude the possibility that FcγR deficiency altered the localization of aPL-IgG, we performed immunohistochemical analyses of deciduas from FcγR–/– and FcγR–/– at day 8 of pregnancy (harvested 60 minutes after treatment with aPL-IgG). Comparable amounts of human IgG were present in FcγR–sufficient and FcγR–deficient mice (Figures 1, b and e). Thus, in our murine model of APS, aPL-IgG targeted to the placenta can initiate fetal damage in the absence of activating FcγRs, while F(ab)′_2 fragments of aPL-IgG do not mediate such injury.

**Figure 2**

C4 or C5 deficiency prevents aPL Ab–induced fetal loss and growth restriction. (a) Pregnant C4+/- and C4–/– mice were treated with aPL-IgG (aPL) (10 mg, intraperitoneally) or NH-IgG on days 8 and 12 of pregnancy, and fetal resorption frequencies were determined on day 15 (n = 5 mice/group). *P < 0.001, aPL versus control. (b–d) Pregnant C5+/- and C5–/– mice were treated intraperitoneally with aPL-IgG (10 mg), monoclonal human aPL Ab (1 mg), monoclonal human anti-DNA (α-DNA; 1 mg), or their respective controls (NH-IgG or monoclonal human anti-rabies Ab, α-R) on days 8 and 12 of pregnancy. Fetal resorption frequencies and fetal weights were determined on day 15 of pregnancy (n = 5–11 mice/group). (b and c) C5–/– mice were protected from fetal loss (b) and growth restriction (c), whereas in the C5+/- mice background strain aPL-IgG or aPL mAb caused pregnancy complications. *P < 0.01, aPL versus control. (d) Day 15 fetuses from C5–/– and C5+/- mice treated with aPL-IgG. Scale bar: 1 cm.
C5 deficiency limits inflammation, necrosis, and activation of C3 by aPL Ab’s. Pregnant C5<sup>+/+</sup> and C5<sup>−/−</sup> mice were treated with aPL-IgG (a, b, d, and e) or NH-IgG (c and f) as described in the legend to Figure 2, and immunohistochemical analysis was performed on decidual tissue from day 8 of pregnancy. (a–c) Detection of C3 in day-8 decidua from aPL-IgG– and NH-IgG–treated mice. The decidua were stained with anti-mouse C3, the chromogen was DAB (brown), and the counterstain was hematoxylin. Decidua of C5<sup>−/−</sup> mice (a) had extensive C3 deposition (arrows), inflammatory cell infiltrates, and necrotic fetal debris, whereas embryos from C5<sup>−/−</sup> mice (b) treated with aPL-IgG appeared normal, and there was limited C3 deposition in decidua at the maternal-fetal interface compared with that of C5<sup>+/+</sup> treated with NH-IgG (c). Original magnification was ×50. (d–f) Detection of human IgG in decidua. Sections were stained with goat anti-human IgG, the chromogen was DAB (brown), and the counterstain was hematoxylin. Within 60 minutes of administering aPL-IgG, human IgG was detectable in decidua from C5<sup>−/−</sup> mice (d) and C5<sup>+/+</sup> mice (e), whereas no IgG was detected in decidua from C5<sup>−/−</sup> mice treated with NH-IgG (f). Data are representative of observations from three to six mice in each experimental group. Original magnification was ×200.

cascade through either the classical or lectin pathways. That the classical pathway is required as an initiator of complement activation by aPL-IgG is supported by our finding that F(ab)′<sub>2</sub> fragments of aPL-IgG, which lack the Fc portion necessary to activate the classical pathway, do not cause pregnancy loss (Figure 1a).

Following initiation of the complement cascade, any of several complement activation fragment-derived ligand-receptor interactions could mediate fetal injury such as those that we have observed. To define which elements of the complement cascade mediate pregnancy loss, we initially focused on complement component 5. C5 is a pivotal member of the complement system because all three initiating pathways converge on C5 and two effector pathways lead from it. To determine whether activation of C5 is required for aPL Ab–induced fetal loss, we treated pregnant C5-deficient and C5-sufficient mice with aPL-IgG, control IgG, human aPL mAb, human anti-DNA mAb, or control human IgG1 mAb. In C5<sup>+/+</sup> mice, both APS patient-derived polyclonal aPL-IgG and human aPL mAb caused a fourfold increase in the frequency of fetal resorption and a significant decrease in embryo weight as compared with control IgG (Figure 2, b–d). Treatment with anti-DNA Ab, an autoantibody often present in patients with APS, had no effect on pregnancy outcome. That the results from experiments with aPL Ab’s and implicates C5 activation and its downstream effects in amplifying local C3 deposition.

As an alternative strategy to confirm that C5 activation is required for fetal loss, we investigated the outcome of blocking C5 activation with anti-C5 mAb (25). These experiments may prove particularly relevant because a similar anti-human C5 mAb is in phase II studies in patients with rheumatoid arthritis and phase I studies in patients with active lupus nephritis (33, 34). We administered anti-C5 mAb before treatment with either NH-IgG or aPL-IgG. The ensuing blockade of C5 cleavage (35) prevented aPL Ab–induced pregnancy loss and growth restriction (Figure 4, a and b). Indeed, pregnant mice treated with anti-C5 mAb were protected to an extent similar to C5-deficient mice (Figure 2, b and c, and Figure 4). C5a-C5aR interactions are critical mediators of aPL Ab–induced pregnancy complications. Two complement effector pathways are initiated by cleavage of C5: C5a, a potent anaphylatoxin and cell activator, and C5b, which leads to formation of the C5b-9 membrane attack complex (MAC). We used two methods to distinguish the role of C5a and the C5aR from that of MAC caused by C5b. First, we treated pregnant mice that had received aPL-IgG with a highly specific peptide antagonist of C5aR, AcPhe-L-ornithine-Pro-

cyclohexylalanine-Trp-Arg, which possesses potent in
vivo anti-inflammatory activities in murine models of endotoxic shock, renal ischemia-reperfusion injury, and the Arthus reaction (26, 27, 36, 37). Administration of C5aR antagonist peptide prevented aPL-Ab–induced pregnancy loss and growth restriction, but had no effect on either frequency of fetal resorption or fetal size in the absence of aPL Ab’s (Figure 5, a and b). Fetal protection conferred by the C5aR antagonist was comparable to that seen with anti-C5 mAb and in mice lacking C5 (Figures 2 and 4), suggesting that downstream pathogenic effects are mediated predominantly by C5a-C5aR interactions. Immunohistological analysis of decidual tissue from mice treated with aPL-IgG and C5aR antagonist peptide yielded results similar to those in C5−/− mice. There was minimal C3 deposition surrounding normal-appearing fetuses and no evidence of inflammation.

As a second approach to test the hypothesis that C5a-C5aR interactions mediate aPL-induced pregnancy complications, we performed studies in mice deficient in C5aR. In the background strain, C5aR+/+, there was a fivefold increase in the frequency of fetal resorption after treatment with aPL-IgG (Figure 5c), whereas, as predicted by experiments with the C5aR antagonist
peptide, aPL-IgG did not increase the frequency of fetal resorption in C5aR–/– mice (Figure 5c). The protective effects of the total absence of C5aRs were also observed when fetal weights were examined (Figure 5c). Taken together, our experiments with C5aR–/– mice and C5aR antagonist peptide identify the C5a-C5aR interaction as a critical effector of aPL Ab–induced injury.

Depletion of neutrophils protects against aPL-induced pregnancy complications. C5a is a potent chemotactic factor and activator of neutrophils. Since we observed neutrophil infiltration at sites of fetal resorption and demonstrated that the C5a-C5aR interaction is necessary for aPL Ab–induced pregnancy loss, we hypothesized that neutrophils were the critical cellular effectors of fetal damage. Indeed, neutrophils have been implicated as effectors in pathogenic Ab-induced arthritis and in Ab-independent murine models of pregnancy loss (38, 39). To examine the relative importance of these cells in aPL Ab–initiated damage, we depleted neutrophils on day 7 of pregnancy by treating mice with rat anti-Gr RB6-8C5; IgG2b Ab served as the isotype control. In the absence of neutrophils, treatment with aPL-IgG did not cause pregnancy loss or growth restriction, nor were there inflammatory infiltrates within the deciduas (Figure 6, a–e). In deciduas from mice treated with anti-Gr plus aPL-IgG (c) there were intact embryos (E) and no inflammatory infiltrates, while in deciduas from mice treated with aPL-IgG plus IgG2b (d) there was extensive neutrophilic infiltration (stained with anti-Gr shown in e) surrounding embryonic debris (ED). Original magnification was x400. (e) Immunohistochemistry to detect infiltrating granulocytes in decidual tissue from an aPL-IgG plus IgG2b–treated mouse. Original magnification was x1,000. (f and g) Immunohistochemistry for C3 deposition in decidua from aPL-IgG–treated mice. Staining for C3 (arrows) was less intense and limited to the fetal-maternal interface in deciduas from mice that had received anti-Gr before treatment with aPL-IgG (f), compared with that of mice treated with aPL-IgG plus IgG2b (g). In the presence of infiltrating neutrophils, C3 deposits were present throughout decidual tissue (g), with particularly intense staining at the fetal-maternal interface surrounding the necrotic residual embryonic debris (arrows). Original magnification was x400.

Figure 6
Neutrophil depletion protects mice from aPL Ab–induced pregnancy complications and limits C3 deposition. BALB/c mice received antimouse granulocyte RB6-8C5 mAb (anti-Gr) (100 µg, intraperitoneally) or IgG2b isotype control mAb on day 7 of pregnancy. On days 8 and 12, mice were treated with aPL-IgG or NH-IgG (n = 5–11 mice/group). (a) and (b) Neutrophil depletion protected mice from fetal resorption (*P < 0.01, aPL-IgG plus anti-Gr versus aPL-IgG plus IgG2b) and (b) growth restriction (*P < 0.01, aPL-IgG plus anti-Gr versus aPL-IgG plus IgG2b). (c and d) Histologic sections of deciduas from day 8 of pregnancy were stained with H&E. In deciduas from mice treated with anti-Gr plus aPL-IgG (c) there were intact embryos (E) and no inflammatory infiltrates, while in deciduas from mice treated with aPL-IgG plus IgG2b (d) there was extensive neutrophilic infiltration (stained with anti-Gr shown in e) surrounding embryonic debris (ED). Original magnification was x400. (e) Immunohistochemistry to detect infiltrating granulocytes in decidua from an aPL-IgG plus IgG2b–treated mouse. Original magnification was x1,000. (f and g) Immunohistochemistry for C3 deposition in decidual tissue. Staining for C3 (arrows) was less intense and limited to the fetal-maternal interface in deciduas from mice that had received anti-Gr before treatment with aPL-IgG (f), compared with that of mice treated with aPL-IgG plus IgG2b (g). In the presence of infiltrating neutrophils, C3 deposits were present throughout decidual tissue (g), with particularly intense staining at the fetal-maternal interface surrounding the necrotic residual embryonic debris (arrows). Original magnification was x400.
consumption by IgG-opsonized neutrophils. Rather, our results are consistent with the conclusion that neutrophils contribute directly to fetal injury. Thus, while among its many effects C5a can activate platelets, endothelial cells, and mononuclear phagocytes, it appears that C5a-mediated recruitment (and likely activation) of neutrophils in the placenta is critical for the development of pregnancy loss and fetal damage.

Alternative pathway of complement activation contributes to aPL Ab–induced fetal loss. In the absence of neutrophil infiltration in decidual tissue, whether as a consequence of blockade of C5a-C5aR interactions or neutrophil depletion, we observed limited activation of C3 and improved pregnancy outcomes (Figure 3, b and e, and Figure 6, c and f). It has been suggested that neutrophils promote complement deposition by causing tissue damage that triggers complement activation and by secreting C3 and/or properdin at sites of inflammation to amplify complement activation via the alternative pathway (40, 41). Given the importance of neutrophils in our model of APS and their potential role as activators of the alternative pathway, we examined the contribution of this pathway of complement activation in aPL Ab–induced pregnancy loss by performing studies in mice deficient in FB. We found that $\beta^{-/-}$ mice were protected from fetal resorption (*$P < 0.05$, $\beta^{-/-}$ aPL-IgG versus NH-IgG) and growth restriction ($P < 0.001$, $\beta^{-/-}$ aPL-IgG versus NH-IgG). (c–e) Immunohistochemistry for C3 deposition in decidual tissue from day 8 of pregnancy following aPL-IgG administration. In deciduals from $\beta^{-/-}$ mice treated with NH-IgG (c), there was minimal C3 deposition and an intact embryo (E). In $\beta^{-/-}$ mice treated with aPL-IgG (d), C3 deposits were present throughout decidual tissue surrounding the necrotic residual embryonic debris (arrows). In contrast, in $\beta^{-/-}$ mice treated with aPL-IgG (e), C3 deposition was limited (arrows) and the embryos remained intact (E). (f) Detection of C3 by Western blotting. Lysates from deciduals of $\beta^{-/-}$ mice and $\beta^{-/-}$ mice were resolved by electrophoresis and blotted with anti-mouse C3 Ab. C3 deposition was greater in deciduals from aPL-IgG-treated $\beta^{-/-}$ mice than in $\beta^{-/-}$ mice, as evidenced by the presence of the cleaved C3-α′ chain.

In summary, our results show that factor B, C3, C5, and C5aR are required for pregnancy complications triggered by aPL Ab’s and that neutrophils are critical effector cells in our model of APS. That aPL-IgG can initiate fetal damage in the absence of activating FcγRs, but not in the absence of C4, and that F(ab)′2 fragments of aPL-IgG do not mediate such injury, suggest that initiation of the complement cascade occurs via the classical pathway. Our observation that factor B is required for fetal death and that its presence is associated with increased C3 deposition...
shows, however, that the alternative pathway amplifies local complement activation and also plays a critical role in the induction of fetal loss.

Discussion
We have shown in a murine model of APS induced by passive transfer of human aPL Ab's that complement activation plays an essential and causative role in fetal loss and tissue injury and, in contrast to other models of Ab-mediated disease, that activating FcγRs are not required for aPL Ab–induced effects. Specifically, we have identified the proinflammatory sequelae of C5a-C5aR interactions and the recruitment of neutrophils as the critical intermediates linking pathogenic aPL Ab's to fetal damage. Our conclusions are based on the fetal protective effects of C5aR deficiency and C5aR antagonist peptide, the similar findings with anti-C5 mAb and in C5−/− mice, where C5a generation is prevented, and on the effects of neutrophil depletion.

Our observations that C4+/− mice are protected from aPL Ab–induced pregnancy loss and that F(ab)′2 fragments of aPL-IgG do not cause fetal injury indicate that the classical pathway is the initiator of complement activation and is required for tissue damage. Generation of C5a, through activation of the classical complement pathway, amplifies the effects of aPL Ab's targeted to the placenta. C5a attracts and activates neutrophils, monocytes, and mast cells, and stimulates the release of inflammatory mediators, including reactive oxidants, proteolytic enzymes, chemokines, cytokines, and complement factors C3 and properdin. Secretion of C3 and properdin by neutrophils, as well as the presence of apoptotic and necrotic decidual tissue, may accelerate alternative pathway activation (dashed line), creating a proinflammatory amplification loop at sites of leukocyte infiltration that enhances C3 activation and deposition and generates additional C5a. This results in further influx of neutrophils, inflammation within the placenta, and, ultimately, fetal injury. Depending on the extent of damage, either death in utero or fetal growth restriction ensues. PMN, neutrophil; Mθ, monocyte/macrophage.

Figure 8
Mechanism of aPL Ab–induced fetal damage. APL Ab’s are preferentially targeted to the placenta where they activate complement via the classical pathway leading to the generation of potent anaphylatoxins and mediators of effector cell activation, particularly C5a. C5a attracts and activates neutrophils, monocytes, and platelets and stimulates the release of inflammatory mediators, including reactive oxidants, proteolytic enzymes, chemokines, cytokines, and complement factors C3 and properdin. Secretion of C3 and properdin by neutrophils, as well as the presence of apoptotic and necrotic decidual tissue, may accelerate alternative pathway activation (dashed line), creating a proinflammatory amplification loop at sites of leukocyte infiltration that enhances C3 activation and deposition and generates additional C5a. This results in further influx of neutrophils, inflammation within the placenta, and, ultimately, fetal injury. Depending on the extent of damage, either death in utero or fetal growth restriction ensues. PMN, neutrophil; Mθ, monocyte/macrophage.
Because apoptotic and necrotic cells activate alternative and classical pathways, neutrophil-induced cell damage may in and of itself increase C3 deposition in decidual tissues (53). In addition, neutrophils can enhance complement activation by releasing complement components, including C3 and properdin, a critical positive regulator of the alternative pathway. Properdin functions by stabilizing the interaction of fB with complement components, including C3 and properdin, a critical positive regulator of the alternative pathway. Properdin functions by stabilizing the interaction of fB with the plasma membrane. In a rat model of Ab-mediated thrombotic glomerulonephritis, C5aR blockade prevents thrombus formation and leukocyte accumulation, and, similar to our findings, depletion of neutrophils prevents glomerular thrombosis, despite the presence of C3 and MAC (52). These studies underscore the link between complement activation and thrombophilia in inflammatory diseases.

One of our most striking findings is that C3 deposition in decidual tissue of aPL Ab-treated mice is diminished in the absence of C5 activation and C5a release. We observed this phenomenon in mice treated with anti-C5 mAb, mice lacking C5 or C5aR, and mice treated with C5aR antagonist peptide. While decreased C3 deposition as a consequence of C5 activation blockade may appear counterintuitive because C3 activation precedes C5 activation, we believe this finding is explained by the coincidental inhibition of neutrophil infiltration. In each setting where C5 activation was blocked, neutrophils were absent from decidual tissues, and in neutrophil-depleted mice, C3 deposition was substantially decreased. Thus, C3 activation and deposition do not appear to be solely dependent on complement components because these are ample in the plasma and extracellular fluid; rather, in the absence of neutrophils, there is limited amplification of the cascade and cleavage of C3.

Because apoptotic and necrotic cells activate alternative and classical pathways, neutrophil-induced cell damage may in and of itself increase C3 deposition in decidual tissues (53). In addition, neutrophils can enhance complement activation by releasing complement components, including C3 and properdin, a critical positive regulator of the alternative pathway. Properdin functions by stabilizing the interaction of fB with spontaneously generated initial C3(H2O) and the formation of the C3 convertase C3bBb (40, 41). Such positive regulatory activity permits properdin to significantly enhance alternative pathway C3 activation resulting either from initiation of this pathway directly by C3(H2O) formation or indirectly through the amplification loop, which uses C3b generated from the classical pathway C3 convertase C4b2a. Thus, properdin and C3 secretion by neutrophils may accelerate alternative pathway activation at sites of leukocyte infiltration, enhancing C3 activation and deposition (40). Our results suggest that initial C3 deposition catalyzed by classical pathway activation leads to C5a generation, attracting neutrophils and potentially triggering properdin release (Figure 8). Furthermore, the experiments in the fB−/− mice support the possibility that properdin and the alternative pathway generate most C3 at sites of injury and initiate a positive feedback loop that generates additional C5a (Figure 8).

The linkage of alternative pathway activation with neutrophil infiltration may also account for the resistance of mice deficient in fB, C3, C5/C5aR, or neutrophils to joint damage after treatment with arthritogenic Ab's, a phenotype that parallels our model (38, 45, 46, 54, 55). There are, however, fundamental differences in the mechanisms of tissue damage in these two experimental models of Ab-mediated injury. Arthritogenic Ab's act through both FcγR and C5a, the latter generated exclusively through the alternative complement pathway with classical pathway components entirely dispensable (38, 46). In contrast, our studies clearly show that fetal injury caused by aPL Ab's requires the classical complement pathway as an initiator and is independent of FcγR. Nonetheless, a common and unexpected finding emerged in both experimental models—the importance of the alternative pathway for injury. Our findings are novel in that they link alternative pathway activation to neutrophil infiltration and raise the possibility that infiltrating cells regulate local complement activation.

That blockade of C5 or C5aR is effective in preventing fetal injury in APS has important therapeutic implications. Blocking the complement cascade at C5 inhibits mediators and effectors of tissue injury while preserving the complement-derived immunoprotective functions of C3. Complement inhibitors are now being tested in patients with inflammatory, ischemic, and autoimmune diseases. Identifying complement-related markers that predict high risk for fetal loss will allow us to translate insights about the mechanisms of complement-mediated disease to interventions that may prevent, arrest, or modify the deleterious effects of aPL Ab's.

**Acknowledgments**

This research was supported by the Alliance of Clinical Research (J.E. Salmon and V.M. Holers), Mary Kirkland Center for Lupus Research (J.E. Salmon), S.L.E. Foundation Inc. (J.E. Salmon), National Kidney Foundation (J.M. Thurman), and NIH grants AI-31105 (to V.M. Holers), AI-25011 (to R.A. Wetsel), and GM-62134 (to J.D. Lambris).


