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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 FcγRs 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 APS and provide novel and important targets for prevention of pregnancy loss in APS.


Introduction

Antiphospholipid syndrome (APS) is characterized by thrombosis and pregnancy loss that occur in the presence of antiphospholipid (aPL) Ab’s (1). Over the last two decades, APS has emerged as a leading cause of pregnancy loss and pregnancy-related morbidity. It is now recognized that recurrent miscarriage occurs in 1% of couples (2–4) and that up to 20% of women with recurrent miscarriage have aPL Ab’s. In the majority of these otherwise normal women, aPL Ab’s are the sole explanation for fetal loss (5, 6). The primary treatment for these patients, anticoagulation throughout pregnancy, is fraught with potential complications, including hemorrhage and osteoporosis. Moreover, treatment can prove to be ineffective. Identification of the mechanisms of pregnancy loss in women with aPL Ab’s would permit development of safer and more effective therapies. Here we identify blockade of the receptor for a proteolytic fragment of complement component 5a (C5a) as a particularly effective treatment in mice and a potentially important target for treatment of patients.

Several murine models have been developed to study mechanisms of fetal loss in APS. In one model, passive transfer of human IgG isolated from aPL Ab–positive sera from women with recurrent fetal loss, as well as murine and human monoclonal aPL Ab’s, induce fetal loss and growth restriction in pregnant mice, demonstrating the direct pathogenic role of aPL Ab’s (7–10). While the specific antigenic reactivity of aPL Ab’s is critical for their effects, the pathogenic mechanisms that mediate aPL Ab–induced vascular thrombosis, tissue injury, and recurrent fetal loss remain incompletely understood (11–14).

We hypothesized that complement activation is a necessary in vivo intermediary step for the clinically relevant deleterious effects of aPL Ab’s on endothelial and inflammatory cells, platelets, and trophoblast cells.
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 effectors 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 pathogenic 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-H2-2H2-T18c Hc/o2Sn) and the C5−/− background-strain mice (B10.D2-H2H2-T18c Hc/o2Sn) and the C5−/− background-strain mice (B10.D2-H2H2-T18c Hc/o2Sn) 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−/− littermates were used for studies. Mice deficient in factor B (B−/−) were generated by targeted deletion (21). The B−/− mice were generated by intercrossing of B−/− and then maintained as a homozygous strain. Procedures that involved mice were approved by the local Committee on Animal Use in Research and Education and were conducted in strict accordance with guidelines for the care and use of laboratory research animals promulgated by the NIH.

Preparation of aPL and other Ab's. Human IgG containing aPL Ab's (aPL-IgG) were obtained from three patients with APS characterized by high-titer aPL Ab's (>140 GPL units), thromboses, and/or pregnancy losses (1). IgG was purified by affinity chromatography using protein G-Sepharose chromatography columns (Amersham Pharmacia Biotech, Piscataway, New Jersey, USA). Human IgG from healthy non-autoimmune individuals (NH-IgG) was purified by an identical method. All IgG samples were treated to deplete endotoxin with Centriprep ultracentrifugation devices (Millipore Corp., Bedford, Massachusetts, USA) and determined to be free of endotoxin using the limulus amebocyte lysate assay. F(ab′)2 fragments were obtained by digestion of purified aPL-IgG pooled from patients 2 and 3 using immobilized pepsin (Pierce Chemical Co., Rockford, Illinois, USA). The digested supernatants were passed through protein G-Sepharose to remove remaining intact IgG, and their purity was assessed by Western blot analysis using an Ab specific for the F(ab′)2 fragment (Jackson ImmunoResearch Laboratories Inc., West Grove, Pennsylvania, USA). The F(ab′)2 fragments were demonstrated to have aPL reactivity similar to the intact aPL-IgG by using ELISA (Sigma-Aldrich, St. Louis, Missouri, USA). The generation, structure, and specificity of the human IgG1 mAb's aPL (mAb S19), anti-DNA (mAb 412.67), and anti-rabies virus (mAb 57) were previously described (10, 22–24).

**Murine pregnancy model.** Females were mated with previously isolated males. The presence of a vaginal plug defined day 0 of pregnancy. On days 8 and 12 of pregnancy, mice were treated with intraperitoneal injections of aPL-IgG (10 mg), aPL-IgG F(ab′)2 (10 mg), NH-IgG (10 mg), and human mAb's (aPL, anti-DNA, or anti-rabies) (1 mg) (7, 17). To inhibit C5, mice were treated on days 8 and 10 of pregnancy with anti-C5 mAb (1 mg, intraperitoneally) or murine IgG as a control (25). To block C5aR, mice received a C5aR antagonist peptide (AcPhe-L-ornithine-Pro-D-cyclohexylalanine-Trp-Arg) (50 µg) on day 8, 30 minutes before treatment with aPL-IgG (26, 27). To deplete neutrophils, mice were treated on day 7 with 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.** Deciduas 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-
Activating FcγRs are not required for aPL Ab–mediated pregnancy loss. Pregnant FcRγ+/+ and FcRγ−/− mice were treated with IgG from a 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+/+. *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 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 control mice (b) or aPL-IgG1 (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 (Fcγ–/–) that is required for signaling by activating FcγRs, high-affinity FcγRI, and low-affinity FcγRIII (18). Although Fcγ–/– deficient mice are reported to have less-severe or undetectable Ab-dependent experimental hemolytic anemia, thrombocytopenia, and glomerulonephritis (32), we found that Fcγ–/– mice were not protected from poor pregnancy outcomes after passive transfer of aPL-IgG (Figure 1a). To exclude the possibility that Fcγ deficiency altered the localization of aPL-IgG, we performed immunohistochemical analyses of deciduas from Fcγ–/– and Fcγ–/– at day 8 of pregnancy (harvested 60 minutes after treatment with aPL-IgG). Comparable amounts of human IgG were present in Fcγ–/–-sufficient and Fcγ–/– deficient mice (Figures 1, b and c). 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.

Blockade of C4 or C5 activation protects mice from aPL Ab–induced pregnancy loss. The complement pathway presents a second Fc-dependent means of effecting Ab-mediated injury, and our initial studies showed that blocking C3 prevents fetal loss in murine APS (17). To assess the importance of the classical pathway of complement activation, we treated pregnant C4–/– mice with aPL-IgG. C4–/– mice were protected from fetal loss (Figure 2a) and growth restriction (average fetal weight in aPL-IgG–treated mice: C4+/+ versus C4–/– 248 ± 19 mg versus 413 ± 30 mg; \( P < 0.001 \), \( n = 5 \) mice/group), suggesting that aPL Ab's trigger the complement

![Figure 2](image-url)

**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.
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)2 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 the C5 attack complex (MAC). We used two methods to distinguish the role of C5a and the C5aR from that of C5b, a potent anaphylatoxin and cell activator, and C5b, an effector for the induction of fetal loss by aPL Ab’s. As an alternative strategy to confirm that C5 activation is a critical proximal effector for the induction of fetal loss by aPL Ab’s and implicate C5 activation and its downstream effects in amplifying local C3 deposition.

In contrast to C5+/+ mice, mice lacking C5 were protected to an extent similar to C5-deficient mice (Figure 2, b and c). Indeed, pregnant mice treated with anti-C5 mAb were similar to results obtained with polyclonal aPL Ab’s also indicates that Ab’s reactive with aPL, rather than xenoreactive Ab’s that may be present in polyclonal human IgG, are sufficient to initiate complement activation and fetal damage in this model.

Figure 3

C5 deficiency limits inflammation, necrosis, and activation of C3 by aPL Ab’s. Pregnant C5+/+ and C5−/− 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 deciduals from aPL-IgG– and NH-IgG–treated mice. The deciduas were stained with anti-mouse C3, the chromogen was DAB (brown), and the counterstain was hematoxylin. Decidua from C5+/+ mice (a) had extensive C3 deposition (arrows), inflammatory cell infiltrates, and necrotic fetal debris, whereas embryos from C5−/− mice (b) treated with aPL-IgG appeared normal, and there was limited C3 deposition in deciduas at the maternal-fetal interface compared with that of C5+/+ treated with NH-IgG (c). Original magnification was ×50. (d–f) Detection of human IgG in deciduas. 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 deciduas from C5+/+ mice (d) and C5−/− mice (e), whereas no IgG was detected in deciduas from C5−/− mice treated with NH-IgG (f). Data are representative of observations from three to six mice in each experimental group. Original magnification was ×200.
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 Figure 4

Figure 4
Inhibition of C5 activation with anti-C5 mAb prevents aPL Ab–induced pregnancy complications. (a and b) Pregnant BALB/c mice were treated with aPL-IgG (10 mg, intraperitoneally) or NH-IgG (10 mg, intraperitoneally) at days 8 and 12 of pregnancy. Mice also received either anti-C5 mAb (1 mg, intraperitoneally) or control murine IgG (Ctrl-mAb; 1 mg, intraperitoneally) on days 8 and 10 (n = 5–11 mice/group). Pregnancies were assessed as described in the legend for Figure 1. Administration of anti-C5 mAb prevented fetal resorption (a) and growth restriction (b). *P < 0.05 versus NH-IgG plus Ctrl-mAb.

Figure 5
Blockade of C5a-C5aR interaction protects pregnancies from aPL Ab–associated injury. (a and b) Pregnant BALB/c mice were given aPL-IgG (10 mg, intraperitoneally) or NH-IgG (10 mg, intraperitoneally) on day 8 and 12, and some also received C5aR antagonist peptide (C5aR-AP) (50 µg, intraperitoneally) on day 8, 30 minutes before administration of aPL-IgG (n = 5–11 mice/group). Pregnancy outcomes were assessed as described in the legend for Figure 1. Treatment with C5aR-AP prevented fetal loss and growth inhibition. C5aR–/– mice were protected from aPL-IgG–induced fetal resorption and growth inhibition. †P < 0.05, aPL-IgG versus NH-IgG.
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 decidua (Figure 6, a–e). Furthermore, without neutrophil infiltration (Figure 6, c–e) there was less C3 deposition, as shown in Figure 6f compared with Figure 6g by grading intensity of C3 staining in embryos and decidua from aPL-IgG–treated mice (anti-Gr versus IgG2b: 2.4 ± 0.7 versus 4.3 ± 0.6; *P < 0.001, n = 5 mice/group). These findings are similar to the limited C3 deposition we observed in C5−/− mice and in C5aR blockade.

To exclude the possibility that neutrophil depletion due to treatment with anti-Gr mAb caused complement consumption, we measured circulating functional C3 levels using a zymosan activation assay before and after treatment with anti-Gr mAb (n = 4 mice) (31). Functional C3 measured at nine time points from 6 to 32 hours after anti-Gr treatment ranged from 95% to 107% of pretreatment levels. At no point was there evidence for a significant decrease in C3, indicating protection against aPL Ab–induced pregnancy loss afforded by anti-Gr mAb treatment is not due to complement consumption.
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 fB−/− mice were protected from fetal resorption and growth restriction caused by aPL Ab's. The frequency of pregnancy loss in fB−/− mice treated with aPL-IgG was comparable to that observed in mice treated with control IgG (Figure 7a). In contrast, fetal wastage and growth restriction was evident in fB+/+ (background strain) mice treated with aPL-IgG (Figure 7, a and b). Immunohistochemical analyses showed substantially less C3 deposition in decidual tissues and embryos from fB−/− mice treated with aPL Ab's than in fB+/+ mice, as shown by comparing the representative sections in Figure 7, d and e, and by grading intensity of C3 staining in embryos and deciduas (fB−/− versus fB+/+: 2.4 ± 0.5 versus 4.0 ± 0.6; P < 0.001, n = 5–7 mice/group). We confirmed the immunohistochemistry results with analysis of lysates from decidual cells by Western blot analysis probed with anti-mouse C3 Ab. C3 deposition was greater in deciduas from aPL-IgG-treated fB+/+ mice than in fB−/− mice, as evidenced by the presence of the cleaved C3-α′ chain.

Figure 7
The absence of fB protects mice from aPL Ab–induced fetal loss and extensive C3 deposition within deciduas. fB+/+ and fB−/− mice were treated with aPL-IgG (10 mg, intraperitoneally) or NH-IgG (10 mg, intraperitoneally) on days 8 and 12 of pregnancy. Fetal resorption frequencies and fetal weights were determined on day 15 of pregnancy (n = 4–8 mice/group). (a and b) In contrast to fB−/− mice, those deficient in fB were protected from fetal resorption (*P < 0.05, fB+/+ aPL-IgG versus NH-IgG) and growth restriction (P < 0.001, fB+/+ aPL-IgG versus NH-IgG). (c–e) Immunohistochemistry for C3 deposition in decidual tissue from day 8 of pregnancy following aPL-IgG administration. In deciduas from fB+/+ mice treated with NH-IgG (c), there was minimal C3 deposition and an intact embryo (E). In fB−/− mice treated with aPL-IgG (d), C3 deposits were present throughout decidual tissue surrounding the necrotic residual embryonic debris (arrows). In contrast, in fB−/− 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 deciduas of fB+/+ mice and fB−/− mice were resolved by electrophoresis and blotted with anti-mouse C3 Ab. C3 deposition was greater in deciduas from aPL-IgG–treated fB+/+ mice than in fB−/− mice, as evidenced by the presence of the cleaved C3-α′ chain.
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; Mφ, monocyte/macrophage.
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.

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