

resist them. Stretch-related changes in FM physical properties may be a protective mechanism against premature FM rupture.

Our hypothesis that acute repetitive stretch would cause a decrease in rupture strength was disproved. Rather, repeti-

tive stretching causes an *increase* in FM rupture strength.

#### CLINICAL IMPLICATIONS

■ Acute stretch is less important than biochemical processes in causing fetal membrane weakening.

■ The process of fetal membrane weakening and rupture is complex, suggesting that more research is required to understand the process before therapeutic interventions to avoid or repair aberrant rupture are likely to be successful. ■

## Excessive complement activation is associated with placental injury in patients with antiphospholipid antibodies

Jaime M. Shamonki, MD; Jane E. Salmon, MD; Elizabeth Hyjek, MD; and Rebecca N. Baergen, MD

### BACKGROUND AND OBJECTIVE

In pregnancy, circulating antiphospholipid antibodies (APAs) are associated with histopathologic changes in the placenta that reflect decreased uteroplacental perfusion, including villous infarction, decidual vasculopathy, decidual vascular thrombosis, and “accelerated” villous maturity. Although traditional experimental models have emphasized the role of thrombosis in placental tissue, recent studies demonstrate that the trophoblastic basement membrane is a particular target for APAs, which suggests that these antibodies may be directed specifically at the placenta.

### OVERVIEW

Studies that used a murine model of antiphospholipid syndrome have demonstrated a critical role for complement activation that lead to fetal and placental injury in the presence of antiphospholipid antibodies. Using immunohistochemical methods, we examined the placentas of patients with antiphospholipid antibodies for deposition of complement activation products from various pathways in the villous interface between mother and fetus.

that complement activation products recruit and activate inflammatory cells into the placenta and induce injury, either directly or indirectly.

No study to date has demonstrated complement activation in the presence of APAs in the human placenta, in which maternal blood has direct contact with the trophoblast, which exposes fetal tissue to circulating maternal antibodies. The classic pathway of complement activation is mediated by antibody-antigen complexes; therefore, we directed our preliminary investigation in that arena.

In this study, we use immunohistochemical methods to analyze placentas from patients with APAs for the deposition of complement activation products from the classic (C4d), early common (C3b), and terminal common (C5b-9) pathways in the villous interface between mother and fetus.

A murine model has demonstrated a critical role for complement in mediating fetal tissue damage in antiphospholipid syndrome. Passive transfer of human immunoglobulin G from patients with high-titer APAs induces fetal death and/or fetal growth restriction in murine pups. In the placentas, a marked deciduitis and immunohistochemical evidence of APAs and complement deposition are present. Mice deficient in C3, C4, or C5 are protected from fetal death that would ensue normally from exposure to APAs, despite evidence of APAs in the placenta. Similar results are found in APA-exposed mice to which specific inhibitors of C3, C5a, or factor Bb have been administered.

These studies demonstrate that, although the nature of the antigen recognized by APAs directs their deposition, complement activation is crucial for the induction of tissue injury and fetal loss that is induced by APAs. It is suggested

### MATERIALS AND METHODS

Patients were identified by retrospective review of surgical pathologic reports from 2001 to 2004. Control cases were selected on the basis of a clinical history of normal pregnancy and absence of significant placental pathologic findings. The study population included 47 patients with a clinician-provided history of positive APAs.

To better isolate the effect of APAs alone on complement deposition, only placentas of viable term infants from patients with APAs, not with antiphospholipid syndrome, were included in the study. All clinical information was provided by the submitting obstetrician.

From the Department of Pathology, New York Presbyterian Hospital–Weill Medical College (Drs Shamonki, Hyjek, and Baergen), and the Department of Medicine, Hospital for Special Surgery–Weill Medical College (Dr Salmon), Cornell University, New York, NY.

Presented at United States and Canadian Academy of Pathology meetings in San Antonio, TX (February 2005), and Atlanta, GA (February 2006).

Cite this article as: Shamonki JM, Salmon JE, Hyjek E, Baergen RN. Excessive complement activation is associated with placental injury in patients with antiphospholipid antibodies. *Am J Obstet Gynecol* 2007; 196:167.e1-167.e5.

0002-9378/free

© 2007 Mosby, Inc. All rights reserved.

doi: 10.1016/j.ajog.2006.10.879



Download the full-length article at [www.AJOG.org](http://www.AJOG.org)

**TABLE**  
**Comparison of immunohistochemical staining**

Variable	APA cases (n = 47)	Control patients (n = 23)	P value
C4d cytoplasm of villous trophoblast	105.3 ± 34	20 ± 4	<.001*
C4d cellular and basement membrane of villous trophoblast	87.2 ± 59	28.4 ± 25	<.001*
C4d extravillous trophoblast of decidua	98.8 ± 57	42.2 ± 37	<.001*
C3b cytoplasm of villous trophoblast	59.3 ± 36	36.3 ± 28	.005*
C3b extravillous trophoblast of decidua	95.5 ± 55	70.8 ± 55	.090 (NS)
C5b-9 cytoplasm of villous trophoblast	64.5 ± 57	113.8 ± 59	.005*
C5b-9 extravillous trophoblast of decidua	162 ± 22	146 ± 34	.065 (NS)

Data are expressed as mean ± SD. H-scores for C4d, C3b, and C5b-9 in patients with APAs and in normal control patients. (H-score is the  $\Sigma Pi(I)$ , where I is the intensity of the immunostaining.) NS, not significant.

\* Significance at  $P \leq .05$ .

Histopathologic diagnoses were rendered on all placentas by 1 pathologist at the time of specimen submission to pathologic evaluation, independently of this study.

Samples were obtained from surgical pathology files from New York Presbyterian Hospital–Cornell. Tissue samples were processed in formalin and embedded in paraffin. A 5- $\mu$ m-thick full-thickness section of each placenta was prepared for each immunohistochemical stain. Tissue uninvolved by pathologic lesions was selected as more representative of the placental function as a whole and more accurately reflective of dynamic processes. Immunohistochemical staining was performed with monoclonal mouse antibodies anti-SC5b-9, anti-C3b, and polyclonal rabbit anti-human C4d antibody. Appropriate positive and negative isotypic controls, including normal placenta, were used for each antibody.

The intensity of immunoreactivity and percentage of cells that were stained in each case were evaluated for each antibody to calculate an H-score, defined as  $\Sigma Pi(I)$ , where I is the intensity of staining with a value of 0, 1, or 2 (none-to-minimal, moderate, or strong, respectively) and Pi is the percentage of cells that were stained for each intensity, varying from 0–100%, for a maximum score of 200. Scores were recorded for each cell type (cytotrophoblast, syncytiotrophoblast, extravillous trophoblast), location (cytoplasm, cell membrane, basement membrane), and antibody (C3b, C4d,

and C5b-9). When scoring the slides, the pathologist was blinded to cases vs control patients.

## RESULTS

Patients with APAs had significantly more previous spontaneous abortions and, accordingly, greater gravidity (a difference that approached significance). No other clinical differences were observed. Patients were of similar age and parity. The pregnancies of both groups produced placentas and fetuses of similar weights. Because only placentas from full-term viable infants were included in the study, fetuses were of a comparable gestational age.

C4d reactivity was observed in 3 areas within the placenta: villous trophoblast (syncytiotrophoblast and cytotrophoblast) cytoplasm, villous trophoblast cell and basement membrane, and the extravillous trophoblast of the basal plate. Reactivity to C3b and C5b-9 were observed only in the extravillous trophoblast and villous trophoblast cytoplasm.

Immunoreactivity to C4d protein was significantly stronger in the villous trophoblast cytoplasm ( $P < .001$ ), cell and basement membrane ( $P < .001$ ), and extravillous trophoblast ( $P < .001$ ) in APA cases compared with control patients. Significantly greater immunoreactivity to C3b ( $P = .005$ ) was also observed in the villous trophoblast cytoplasm in placentas of patients with APAs, compared with normal control patients. In contrast, we found significantly less deposi-

tion of C5b-9 in the villous trophoblast cytoplasm ( $P = .005$ ) of APA cases vs control patients (Table).

Additionally, we found a significant correlation between the presence of pathologic lesions and the deposition of C4d in the trophoblast cytoplasm ( $P < .001$ ) and cellular and basement membranes ( $P = .001$ ).

## COMMENT

We have documented a novel finding in the placentas of patients with APAs by demonstrating an increased deposition of complement factors C4d and C3b, compared with normal control patients. Even when clinically silent, by measures such as fetal birthweight, APAs produce histopathologic changes in the placenta and, accordingly, are associated with increased complement deposition. As in the murine models of antiphospholipid syndrome, these results support the role of complement in the induction of placental tissue injury in the presence of APAs. Taken together, these studies suggest that proinflammatory factors that stimulate complement activation may precede the changes that ultimately lead to ischemia, tissue injury, and fetal loss.

Complement activation products have the capacity to activate leukocytes and endothelial cells, thereby inducing a prothrombotic phenotype. As such, complement activation may be a critical event that precedes the thrombosis that defines antiphospholipid syndrome. Heparin therapy actually may prevent

APA-associated miscarriage by inhibiting the proinflammatory factors that lead to fetal tissue injury.

A body of evidence has mounted that demonstrates the complement-inhibitory effects of heparin, which may account for its ability to avert miscarriage or fetal morbidity in the presence of APAs. This argument is furthered by Girardi et al, who demonstrated that, unlike heparin, anticoagulants (fondaparinux and hirudin) that do not inhibit complement activation do not prevent pregnancy loss in mice that are exposed to APAs.

An unexpected finding was that we observed a decrease in C5b-9 complement protein in APA cases, compared with control patients. C5b-9, the membrane attack complex, leads to tissue injury through permeabilizing cell membranes and acting as an ion channel to trigger cell activation.

In addition to producing membrane attack complex, activation of the complement common pathway releases the anaphylatoxins C3a and C5a, which are independent inflammatory mediators that are capable of leading to tissue damage. The pathologic condition that is observed in these APA cases correlates significantly with deposition of C4d and may be the effect of anaphylatoxin release (C3a, C5a) rather than because of the direct effects of membrane attack complex.

More specific therapy, namely an agent that specifically inhibits complement activation, may be useful in the future treatment of pregnant patients with APAs. Future studies, to include patients with more severe clinical symptoms and adverse obstetric outcomes, will address specifically the differences in complement deposition between patients with clinical antiphospholipid syndrome vs the presence of APAs alone.

#### CLINICAL IMPLICATIONS

- Even when clinically silent, by measures such as fetal birthweight, antiphospholipid antibodies produce histopathologic changes in the placenta and, accordingly, are associated with increased complement deposition.
- Complement activation products have the capacity to activate leukocytes and endothelial cells and thereby induce a prothrombotic phenotype. As such, complement activation may be a critical event that precedes the thrombosis that defines antiphospholipid syndrome.
- A therapeutic agent that specifically inhibits complement activation may be useful in the future treatment of pregnant patients with antiphospholipid antibodies. ■

## Expression and function of protease-activated receptor 4 in human myometrium

Nicholas M. Allen, MB; Margaret O'Brien, PhD; Anne M. Friel, PhD; Terry J. Smith, PhD; John J. Morrison, MD

### BACKGROUND AND OBJECTIVE

Thrombin is a well-known serine protease that plays an important homeostatic role in blood coagulation. In addition, thrombin exerts nonthrombotic

From the Department of Obstetrics and Gynecology, National University of Ireland, and the Clinical Science Institute, University College Hospital Galway (Drs Allen, Friel, and Morrison), and the National Centre for Biomedical Engineering Science, National University of Ireland (Drs O'Brien, Smith, and Morrison), Galway, Ireland.

Cite this article as: Allen NM, O'Brien M, Friel AM, Smith TJ, Morrison JJ. Expression and function of protease-activated receptor-4 in human myometrium. *Am J Obstet Gynecol* 2007;196:169.e1-169.e6.

0002-9378/free

© 2007 Mosby, Inc. All rights reserved.

doi: 10.1016/j.ajog.2006.09.027

### OVERVIEW

We used reverse transcription polymerase chain reaction studies to explore protease-activated receptor 4 messenger RNA and immunofluorescence studies to investigate protein expression in human myometrium. By using isometric tension recordings, we examined functional effects on contractility.

physiologic and pathophysiologic effects in numerous tissues by cleaving and activating a relatively novel group of protease-activated receptors (PARs). To date, 4 subtypes of PAR have been described (PARs1-4); the relative role of each in thrombin-mediated effects in different tissues varies.

In the rat, the functional presence of PARs in both pregnant and nonpregnant myometrium has been suggested. PAR-1

messenger RNA (mRNA) has been identified in rat myometrium. However, for human myometrial smooth muscle, little is known about the expression or role of PARs in relation to uterine quiescence or excitability. We have recently reported the functional presence of PAR-1 in human myometrium during pregnancy and the nonpregnant state and outlined that PAR-1 activation exerts a significant uterotonic effect.

Data are limited in relation to the expression or potential role of PAR-4 in the human uterus. The aims of this study were (1) to investigate the expression of PAR-4 at the mRNA level in human myometrium, (2) to investigate the expression of PAR-4 protein in smooth muscle cells derived from human myometrium, (3) to evaluate the functional effects of PAR-4 activation and specific PAR-4 antagonism on myometrial contractility, and (4) to examine the role of PAR-4 in thrombin-mediated contractility.



Download the full-length article at [www.AJOG.org](http://www.AJOG.org)