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REVIEW

Complement activation, a threat to pregnancy

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Abstract Pregnancy poses a challenge for the immune systems of placental mammals. As fetal tissues are semi-allogeneic and alloantibodies that commonly develop in the mother, the fetus and the placenta might be subject to complement-mediated immune attack with the potential risk of adverse pregnancy outcomes. Here, I describe how the use of animal models was pivotal in demonstrating that complement inhibition at the fetomaternal interface is essential for a successful pregnancy. Studies in animals also helped the identification of uncontrolled complement activation as a crucial effector in the pathogenesis of recurrent miscarriages, intrauterine growth restriction, preeclampsia, and preterm birth. Clinical studies employing complement biomarkers in plasma and urine showed an association between dysregulation of the complement system and adverse pregnancy outcomes. A better understanding of the role of the complement system in pregnancy complications will allow a rational approach to manipulate its activation as a potential therapeutic strategy with the goal of protecting pregnancies and improving long-term outcomes for mother and child.

Keywords Complement activation · Animal models · Pregnancy complications · Fetal neurodevelopment

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Introduction

For an immunologist, pregnancy remains an enigma. As half the genes are derived from the father, the fetus and the placenta must be considered a 'semi-allograft'. Becoming pregnant is not that different from receiving a transplant from a genetically different individual. Thus, mothers need to adapt to imprinted antigens from the father in order to maintain a successful pregnancy [1, 2]. Although intimate contact with the mother's uterine tissue ensures optimal nourishment and protection of the fetus throughout its early development, it makes the fetus and placenta potential targets for her immune system. The mismatched 'organ transplant' would be quickly rejected without substantial immune suppression [2]. Failure of the placenta to escape maternal immune response can cause pregnancy complications such as miscarriages and preeclampsia [3]. In the majority of cases, the placenta and the fetus avoid the attack of the maternal immune system. Why are the placenta and fetus not rejected in the uterus? Many mechanisms protect the fetus from attack by the maternal immune system. These include the lack of expression of polymorphic classical class I human leukocyte antigen molecules (HLA-A and B) in invasive fetal trophoblasts [4, 5], tryptophan catabolism by the enzyme IDO that suppresses T cell activity [6], the existence of particular NK cells in the uterus that are proangiogenic instead of cytotoxic [7, 8], and a tight regulation of complement system activation [9, 10].

Complement split product C3b is continually deposited on all surfaces in the body that are in contact with plasma. In the absence of natural protective factors, amplification occurs with the deposition of more C3b and other components and generation of anaphylatoxins C3a and C5a that attract phagocytic cells—macrophages and neutrophils—and initiate tissue destruction. Normal host cells are protected from the harmful effects of complement by cell surface complement regulatory

proteins [11]. In humans, decay-accelerating factor (DAF) and membrane cofactor protein (MCP) prevent complement activation by inhibiting C3 and C5 convertases [12]. In other species, including mice, structural and/or functional homologs of these proteins can be found. The mouse protein complement receptor 1-related gene/protein y (Crry/p65, Crry) is a self-protecting complement regulatory proteins. Crry demonstrates cofactor activity for factor I-mediated cleavage of both mouse C3b and C4b. In addition, Crry also exhibits decay-accelerating activity for the classical pathway C3 convertase. Mouse Crry thus uses the specific mechanisms of both human MCP and DAF to inhibit complement activation [13]. Complement inhibition at the fetomaternal interface is crucial for the maintenance of pregnancy (Fig. 1) [14].

We have learned much about the crucial contribution of complement inhibitors to a successful pregnancy from mouse studies in the Molina Lab. The important role of Crry in protecting pregnancies in mice was discovered by serendipity while investigating whether the murine gene, complement receptor-related gene Y (*Crry*), and its product Crry, contributed to the protection of the body from damage inflicted by inflammation [15]. To elucidate Crry function in vivo, Molina's group created *Crry* knockout mice. By mating $Crry^{+/-}$ with $Crry^{+/-}$, the investigators expected that around 25% of the offspring from these mice would be null for *Crry* and thus have no surface inhibition of C3 or C4 activation. Instead they found no homozygous Crry deficiency among the offspring of these mice, all of the animals that inherited two mutant copies of *Crry* died as embryos at ~ 10 days post-conception. Although Crry is not the only membrane complement regulator in mice, it appeared that its absence was sufficient to leave the embryo unprotected against attack from maternal complement [16, 17]. Normal mice express early in pregnancy large amounts of Crry on trophoblast, highlighting the importance of complement inhibition at the feto-maternal interface [17]. That $Crry^{-/-}$ mice could be rescued on a factor B (fB)- or C3-deficient maternal background confirms that $Crry^{-/-}$ embryos die in utero due to an uncontrolled complement attack [18]. These mouse studies emphasize the important role of complement inhibition in the maintenance of pregnancy.

The presence of complement inhibitors in the human placenta is well documented. Most of the studies aimed to investigate the presence of complement regulatory proteins in human placentas were performed either in term placenta obtained after normal birth or early placenta obtained at abortion. In the term placenta from uncomplicated pregnancies, complement inhibitor C4b binding protein (C4BP) was detected in the syncytiotrophoblast, outer layer of the placenta that facilitates exchange of material between the mother and the embryo, and factor H in the tissue stroma [19]. Gene and protein expression of CD46, CD55, and CD59 was demonstrated in the placental extravillous trophoblasts in all three trimesters during normal pregnancies [9, 20, 21]. CD46 expression was demonstrated in the villous cytotrophoblasts, but CD55 and CD59 were inconsistently reported. Interestingly, the placental cells most in contact with the maternal tissue, the syncytiotrophoblast and extravillous trophoblasts, express all three complement inhibitors to provide protection against maternal complement activation.

Despite the presence of complement regulators, trophoblasts also synthesize complement components. Trophoblast cell lines and primary trophoblasts isolated from women secrete C3 and C4 protein and have mRNA for components C6, C7, C8, and C9 [22], providing immune protection against infection. Positive staining for C1q, C9, and C3d was also detected in normal placenta [19, 20]. Implantation, placentation, and the first trimester of pregnancy require a strong inflammatory response while protecting the mother and embryo against infection [23]. In this line, higher plasma anaphylatoxins C3a, C4a, and C5a concentrations were found during normal pregnancy compared to non-pregnant women [24–26]. If complement activation is involved in the pathogenesis of pregnancy complications, levels of complement split products would be expected to be higher than in normal pregnancy and could provide biomarkers to predict pregnancy outcomes. Complement components as biomarkers will be discussed for each pregnancy complication: preterm birth, preeclampsia, and recurrent miscarriages.

Since uncontrolled complement activation can lead to adverse pregnancy outcomes, recent studies suggested that polymorphisms of complement inhibitor genes might be related to abnormal pregnancy outcomes. Polymorphisms of the human

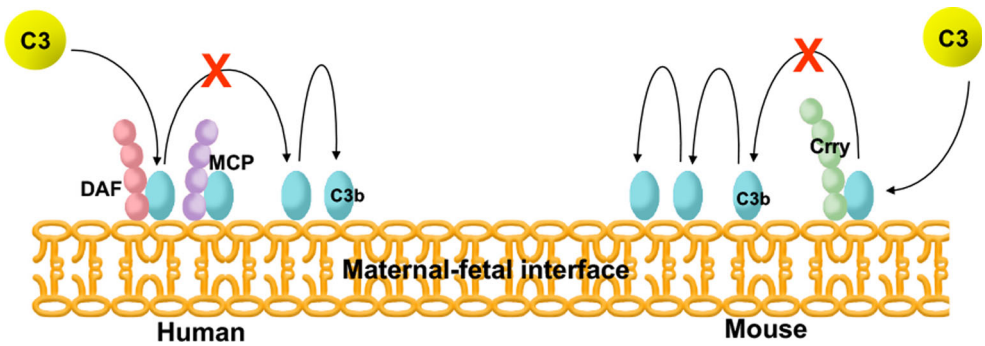

Fig. 1 Appropriate complement inhibition is an absolute requirement for a pregnancy to be successful. Complement inhibitory proteins (MCP and DAF in humans and Crry in rodents) are expressed in the placenta, throughout gestation and control complement activation protecting pregnancies. Figure modified from [16]

CD46 gene in the Caucasian population were observed at increased frequency in females that had unexplained recurrent spontaneous abortions [27–31]. Provocatively, fathers of pregnancies that ended in miscarriage also showed CD46 polymorphisms. Knowing that paternally expressed genes predominate in the placenta, it is tempting to speculate that in pregnancies fathered by an individual carrying a CD46 polymorphism, abnormal placental expression of CD46 might result in excessive complement activation in the placenta and fetal demise. Alterations in the maternal genes encoding for C4b-binding protein were also associated with miscarriages [28]. In contrast, in a study performed in Iran, no genotype differences in CD46 were observed between fertile women and women that experienced recurrent miscarriages [29]. Impaired capacity to limit complement activation was also associated with late pregnancy complications such as preeclampsia. Mutations in CD46, complement factor I, and complement factor H were observed in women with SLE that developed preeclampsia [30]. On the other hand, a study performed in Finland did not find any association between *CD46* SNPs and preeclampsia in women with no autoimmune diseases [31]. The ethnic background and the presence of autoimmunity might be important variables in these studies showing discrepant results. Since the placenta is mainly of paternal/fetal origin, paternal gene expression of complement inhibitors should be investigated and correlated with pregnancy outcomes.

Complement activation is associated with pregnancy complications

Preterm birth

Every year, an estimated 15 million babies worldwide are born preterm (before 37 completed weeks of gestation), and this number is rising. The rate of preterm birth (PTB) ranges from 5 to 18% of babies born in different countries. PTB complications are the leading cause of death among children under 5 years of age, responsible for nearly 1 million deaths in 2015 [32]. The lack of preventive measures to diminish the incidence of PTB and its sequelae is rooted in the lack of knowledge about the normal process of parturition. While term and preterm parturition share a cascade of downstream mediators that participate in cervical ripening and uterine contractions, PTB is a syndrome attributable to multiple pathologic processes. Identification of triggers of PTB might lead to therapeutic strategies to stop the cascade of events leading to premature parturition.

Studies in animals identified a role for complement activation in the pathogenesis of PTB. The association between complement activation and preterm delivery was recognized in mice in which preterm delivery was induced by administration of low-dose endotoxin or the progesterone antagonist RU486 [33]. Administration of both endotoxin and RU486 increased cervical deposition of C3, infiltration of macrophages, and plasma levels of anaphylatoxins compared to gestational age-matched controls. Further, a significant increase in matrix metalloproteinase 9 (MMP-9) activity, collagen degradation, and tissue distensibility were observed in the cervix of mice that received low-dose endotoxin intravaginally [33]. In this study, a significant correlation between C5a and the increased release of MMP-9 by macrophages was observed. Notably, mice deficient in C5a receptors (C5aR) did not show increased MMP-9 activity and cervical remodeling or premature delivery in response to low-endotoxin or RU486, suggesting that the complement system might be a good therapeutic target to prevent PTB [33]. Interestingly, progesterone prevented C5a-induced activation of macrophages by causing internalization of C5aR and reduced PTB in mice [33]. Both complement inhibition and progesterone, which modulates C5aR expression by macrophages, seem to be beneficial therapeutic approaches to prevent premature birth. In this line, progesterone has been approved by the FDA for the prevention of PTB in women with a history of prior spontaneous preterm birth [34]. However, since its approval, contradictory results have been reported [35, 36]. While several studies demonstrated the efficacy of progesterone in preventing PTB [35, 37], a recent trial concluded that vaginal progesterone administration was not associated with reduced risk of PTB or associated neonatal adverse outcomes (death, brain injury, neonatal bronchopulmonary dysplasia, or cognitive score at 2 years of age) [37].

In a follow-up study to investigate the different mechanisms/triggers leading to preterm and term delivery, the role of complement was further elucidated. Contrarily to PTD, complement activation was not required for the physiological process that leads to term delivery in mice. Neither complement deposition in cervical tissue nor increased complement split products in plasma were observed in mice that delivered at term [38]. The fact that mice deficient in different complement components do not have extended gestations confirms that complement does not play a critical role in parturition at term. While both PTD and term delivery showed increased MMPs release, collagen degradation, and cervical remodeling, these common downstream mediators seem to be triggered by different initiators. While activation of complement, in particular C5a, might be the initial step leading to macrophage activation and release of MMPs leading to increased cervical distensibility and preterm birth, MMP release at term is not mediated by complement activation products and originates from cervical fibroblasts and columnar epithelial cells rather than inflammatory cells [38].

That intraamniotic infection (chorioamnionitis) causes premature membrane rupture, and preterm birth is another piece of evidence supporting the role of complement activation in triggering preterm birth. Interestingly, clinical studies showed

a correlation between elevated levels of complement activation fragments Bb and C3a and spontaneous PTB [39, 40]. Even more, it has been suggested that certain complement activation proteins might be biomarkers for PTB; for example, it has been demonstrated that plasma levels of complement factors B and H at 10–15 weeks of gestation are predictive of the development of PTB later in pregnancy [41].

Fetal brain injury associated with preterm birth

Of infants that were born before term, 25 to 50% experience long-term cognitive, behavioral, attentional, or socialization deficits [42, 43]. MRI studies in preterm infants showed decreased volume in the cerebral cortex, suggesting a role for the cortex in these long-term complications [44]. During pregnancy, the developing brain is particularly susceptible to inflammatory insults, often due to maternal intrauterine inflammation/infection. There is growing association between maternal inflammation and fetal brain injury [45, 46]. In this line, a new role for C5a in abnormal fetal brain development in inflammation-induced PTB was demonstrated in mice [47]. C5a not only plays a crucial role in the cervical ripening and myometrial contractions that lead to PTB but also causes damage to the developing fetal brain cortex in the mouse model [47]. Disruption of cortical dendritic and axonal cytoarchitecture characterized by decreased staining for microtubule-associated protein 2 and neurofilament 200 was observed in the brains of fetuses born preterm compared to age-matched controls. Fetuses deficient in C5aR (−/−) did not show cortical brain damage after PTB, suggesting that C5a-C5aR interaction is required for abnormal cortical development in prematurity. Treatment with an anti-C5 antibody that prevents generation of C5a also prevented cortical fetal brain injury in PTB mice [47]. In support of these in vivo studies, C5a also showed a detrimental effect on fetal cortical neuron development and survival in vitro [47]. Isolated cortical neurons from day 16 fetuses extend their neurites and establish synapses becoming mature neurons after 10 days in culture. While long axons were observed in cultured control cortical neurons, the length of axons in C5a-exposed neurons was considerably reduced. The blockade of C5a with C5aR antagonist peptide (C5aR-AP) increased the number of dendrites and the length of axons compared to neurons incubated with only C5a [47]. Increased levels of glutamate were measured in the supernatant of cortical neurons incubated with C5a and addition of the N-methyl-D-asparate antagonist, MK-801 (dizocilpine maleate) prevented C5a and glutamate-induced diminished axonal length, suggesting that C5a neurotoxicity might be mediated by glutamate excitotoxicity. Excitation resulting from stimulation of the ionotropic glutamate receptors is known to cause neuronal apoptosis [48]; indeed, signs of neuronal death/apoptosis and increased release of lactate dehydrogenase were observed in cortical neuron cultures exposed to C5a. This neuronal cell death was prevented by C5aR-AP, confirming the neurotoxic effects of C5a/C5aR [47].

Identification of a biomarker that can non-invasively detect pregnancies at risk of PTB and fetal brain injury during PTB would be of enormous clinical benefit in determining optimal timing of delivery and implementation of neuroprotective strategies. The described animal studies suggest that measuring complement activation might be useful to detect/predict fetuses at risk. Using a novel non-invasive MRI technique, complement activation was identified as a biomarker of abnormal fetal brain development in PTB [49]. During complement activation, C3 activation fragments (C3b/iC3b/C3d) are covalently attached to the injured tissue. Ultrasmall superparamagnetic iron oxide (USPIO, diameter: 5–40 nm) particles can be imaged by magnetic resonance imaging (MRI) because they shorten the T2 and T2* relaxation time; antibodies against C3 activation products conjugated to USPIOs were used in vivo to detect complement activation/inflammation in the fetal brain and predict pregnancy/fetal outcomes in PTB [49]. USPIO-conjugated anti-C3 antibodies crossed the placenta and the fetal blood brain barrier (BBB). Within the fetal sacs, most of the USPIO-conjugated anti-C3 antibodies were found in the placenta and fetal brain (Fig. 2). Increased signal was detected in fetal brains in PTB mice indicating increased C3 deposition [49]. C3 deposition was associated with abnormal cortical cytoarchitecture, increased complement split product C5a levels, and increased neurodegeneration [49].

Figure 3 summarizes the postulated mechanisms responsible for complement induced cervical remodeling, myometrial contraction, and fetal brain injury in a mouse model of preterm birth.

A recent translational study reinforces the hypothesis that C5a is a crucial mediator in fetal brain injury in PTB. In this clinical study, C5a was found in the cerebrospinal fluid of newborn human infants and its levels were elevated in those born preterm [50]. Interestingly, this difference was not explained by systemic infection. This study demonstrates increased complement activation in the brain of neonates born preterm and strengthens the suggestion that complement might be a potential therapeutic target for preterm fetal brain injury.

Preeclampsia

Preeclampsia (PE) affects ~ 5% of pregnancies and is traditionally diagnosed by the combined presentation of high blood pressure and proteinuria. Maternal organ dysfunction such as renal insufficiency, liver involvement, and neurological and hematological complications can also be observed during the onset of PE. PE is associated with uteroplacental dysfunction, diminished placental blood flow, and fetal growth restriction. PE can be lethal, and this disorder is the main cause of maternal and child mortality worldwide.

A

	T2* (ms)
Control (n=8)	32.5±5.5
Preterm (n=5)	31.8±5.0
Control - USPIO (n=4)	32.0±6.6
preterm - USPIO (n=6)	31.8±5.3
Control - USPIOantiC3 (n=6)	32.7±7.9
Preterm - USPIOantiC3 (n=8)	21.9±4.5*
Control - USPIOCtrl Ab (n=4)	30.9±7.1
Preterm - USPIOCtrlAb (n=5)	32.4±5.5

B

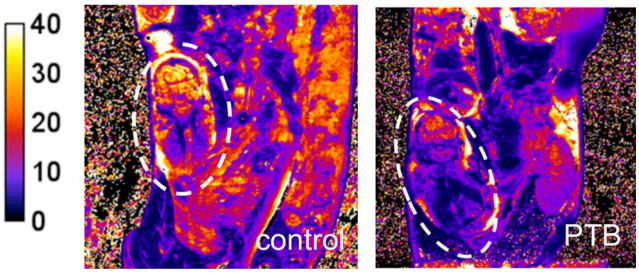

Fig. 2 Significant reduction in T2*-relaxation time, indicative of increased C3 deposition in the fetal brain in PTB mice. A significant reduction in T2* time was observed in vivo in the fetal brains in utero in PTB mice treated with USPIO-antiC3 compared to age-matched control and PTB mice that received USPIOs or control Ab-USPIOs (**a**). *Statistically significant compared to control mice injected with USPIO-antiC3 ($p < 0.05$), to preterm mice that received USPIO ($p < 0.05$) and SPIO-Ctrl antibody ($p < 0.05$) and preterm untreated mice ($p < 0.05$). **b** T2* maps of maternal abdomen in a PTB-mouse and age-matched control injected with USPIO-antiC3 antibodies. The dashed ovals show the amniotic sacs containing the fetuses. Microphotographs represent one of 4–5 similar experiments

Even though PE is predominantly a human disease, with only very rare reports in other species, efforts to develop animal models which mimic the human pathology have yielded inflammatory models that are useful tools to investigate the pathogenic mechanisms of PE and test therapies for prevention and treatment. Animal models of PE have helped establish a role for complement activation in the pathogenesis of this serious pregnancy complication.

The crossing of CBA/J × DBA/2 animals has long been used as a mouse model of spontaneous abortion and PE [51–53]. Along with placental dysfunction, these matings resulted in maternal disease characterized by proteinuria, elevated blood urea nitrogen, and glomerular fibrin deposition and endotheliosis. Like the human disease, pregnancy complications in CBA/J × DBA/2 were associated with a dysregulation in angiogenic factors. Adverse pregnancy outcomes in this model resulted from placental insufficiency and functional deficiency of free vascular endothelial growth factor (VEGF) and elevated levels of soluble VEGF receptor 1 (sFlt-1). Interestingly, Tedesco and Chaouat [54] demonstrated that complement activation in this model was activated primarily through the lectin pathway. Analysis of the implantation sites collected from DBA/2-mated CBA/J mice revealed MBL-A deposition as early as 3.5 days of pregnancy. In addition, MBL-A deficiency prevented pregnancy loss in this abortion-prone mating combination [54]. Inhibition of complement, using Crry-Ig, anti-C5 mAb, or antagonists of C5aR, in vivo reverted the angiogenic imbalance, prevented growth restriction, and rescued pregnancies [53]. In addition, targeted inhibition of complement activation by administration of a chimeric soluble CR2-Crry molecule prevented oxidative stress and placental dysfunction, as well as proteinuria and renal pathologic features of preeclampsia in this model [55]. These findings point to blockade of complement activation as a valuable approach to prevent pregnancy-related complications, including PE and intrauterine growth restriction.

Complement activation has also been linked to pathogenesis in other animal models of PE. In the antibody transfer model of PE, pregnant mice receive autoantibodies that bind and activate the major angiotensin receptor AT1R; a role for complement split product C3a in the development of PE has been shown [56]. Autoantibodies to AT1R activate C3aR resulting in hypertension and proteinuria. Blockade of C3aR attenuated sFlt-1 production of soluble VEGF receptor 1 (sVEGFR-1, also known as sFlt-1; a potent anti-angiogenic molecule), placental dysfunction, and intrauterine growth restriction in this model [56].

In humans, complement activation has also been associated with PE. Complement split products, notably C4d, were identified in focal or diffuse staining patterns in the placentae of women with PE [20]. In addition, mRNA expression for CD55 and CD59 was significantly elevated in the placenta, suggesting a compensatory mechanism to control increased complement activation [20]. Other human studies demonstrated increased C4d deposition in the syncytiotrophoblast in cases of PE with fetal growth restriction [57]. Interestingly, C4d deposition was also observed in placentas from first trimester miscarriages, suggesting that C4d can be a biomarker for at-risk placentas.

Regarding circulating complement split products, an increased C3a/C3 ratio and increased sC5b-9 were observed in preeclamptic pregnancies compared to normal pregnancies [58]. Other studies showed increased C5a plasma levels in pregnancies affected by preeclampsia [59, 60].

Recurrent miscarriages

Animal models of recurrent miscarriages and intrauterine growth restriction (IUGR) also demonstrated that excessive complement activation, particularly C5a generation, is associated with impaired angiogenesis and adverse pregnancy outcomes [52]. Increased C5a levels were associated with deficiency of free vascular endothelial growth factor (VEGF), increased levels of soluble receptor for VEGF 1 (VEGFR-1/sFlt-1), presence of inflammatory infiltrates in the placenta

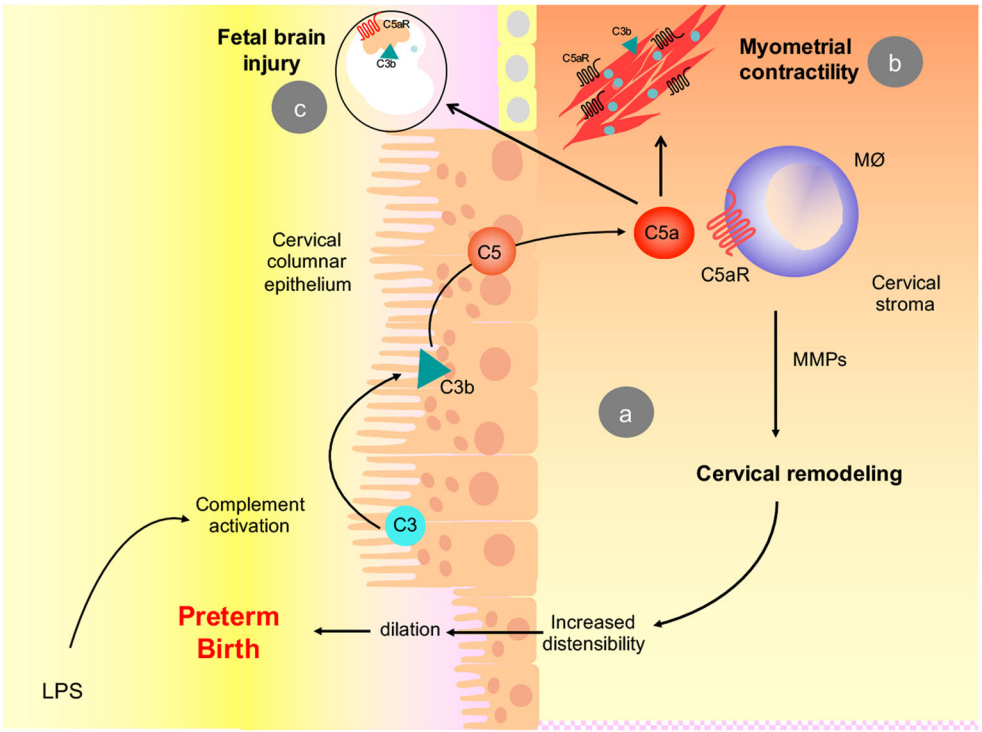

Fig. 3 Role of C5a in cervical remodeling, myometrial contraction, and fetal brain damage in preterm birth induced by intravaginal administration of LPS. **a** C5a attracts and activates macrophages to the cervix. Activated macrophages release metalloproteinases that digest collagen fibers. Cervical remodeling leads to increase distensibility of thr cervix leading to PTB. **b** C5a has uterotonic properties and induces myometrial contractions through interaction with C5aR. **c** C5a affects fetal brain development. Increased neurodegeneration and abnormal architecture are observed in the cortex in fetuses during PTB

and defective placental development in the CBA/J × DBA/2 model of fetal loss. Inhibition of complement activation in vivo has been shown to prevent angiogenic factor imbalance leading to improved pregnancy outcome [53]. In humans, it has been estimated that up to 20% of early pregnancy losses that are not mediated by autoantibodies are associated with hypocomplementemia [61].

Obstetric antiphospholipid syndrome

The antiphospholipid syndrome (APS), characterized by the presence of antiphospholipid (aPL) antibodies, is frequently associated with serious pregnancy complications: recurrent fetal loss, preterm birth, preeclampsia, and IUGR. Thrombosis is also frequently observed. Both pregnancy complications and thrombosis are tightly correlated with excessive levels of complement activation [62–64]. Murine models of OAPS in which pregnant mice are injected with human IgG containing aPL antibodies or mouse aPL monoclonal antibodies were fundamental to uncovering the triggering of the classical pathway by aPL antibodies and to establish C5a and neutrophils as key mediators of fetal injury [63, 65]. Using this model, the crosstalk between the complement system and the coagulation system was underscored, identifying C5a as a stimulus for the expression of the coagulation factor tissue factor (TF) on neutrophils. Through a mechanism that involves TF and protease activated receptor (PAR) expression, C5a activates neutrophils at the fetomaternal interface causing placental injury and leading to fetal death in OAPS [66, 67], Fig. 4. Pravastatin, by downregulating TF and PAR-2, rescued pregnancy in the mouse model of OAPS.

Due to their large size, antibodies are not expected to pass the blood-brain barrier (BBB) under physiological conditions. Provocatively, during pregnancy, aPL antibodies cross the placenta and the BBB reaching the developing brain [68]. The BBB becomes more permeable during inflammation. In particular, C5a regulates BBB integrity in inflammatory settings where it affects both endothelial and astroglial cells [69]. In addition, the immaturity of the fetal BBB, characterized by increased permeability, increases the susceptibility of the fetal brain to maternal autoantibodies and other proinflammatory and toxic insults.

Using in vivo SPECT/CT, it was demonstrated that aPL antibodies labeled with Indium-111 are rapidly cleared from circulation in the pregnant mouse and large amounts of the radiolabeled antibody are entrapped in the placentas and fetal brains within the fetal sacs [68]. This was the first study to show in vivo in real time the passage of aPL antibodies to the fetus targeting the fetal brain [68]. Binding of aPL antibodies to the fetal brain was associated with complement deposition, measured using USPIO-labeled anti C3 antibodies [49]. Interestingly, aPL antibodies bind to the fetal brain and activate the complement cascade leading to abnormal fetal brain architecture and abnormal behavior in the offspring [49].

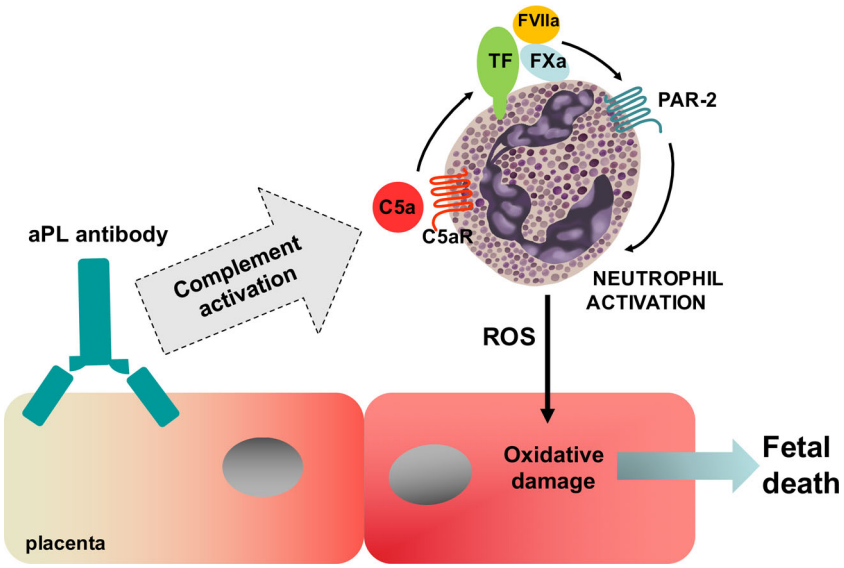

Fig. 4 C5a-induced tissue factor plays a crucial role in placental and fetal injury induced by aPL antibodies. aPL antibodies bind to the placenta and activate the complement cascade. C5a attracts and activates neutrophils to the fetomaternal interface. C5a activates neutrophils through the expression of tissue factor and protease activated receptor-2 (PAR-2). Increased neutrophil activity results in increased oxidative damage of the placenta and fetal loss [66, 67]

Increased anxiety was observed in the offspring of OAPS mice, suggesting that in utero exposure to aPL antibodies causes abnormal cortical development and that C3 activation might be a footprint for adverse fetal outcomes. In conclusion, the pathogenic effects of aPL antibodies on the placenta and the developing brain seem to be mediated by complement activation, suggesting that complement activation products might be useful biomarkers of pregnancy outcomes and targets for therapy. Interestingly, a recent case report described a pregnant patient treated with the C5-inhibitor eculizumab to prevent APS-related complications [70]. No new thrombosis or catastrophic APS developed during the last week of pregnancy or postpartum after C5 inhibition [70]. Another case report showed efficacy of C5 inhibition with eculizumab in the treatment of PE [71]. These case reports suggest that targeting C5 activation might be a safe treatment option for OAPS and PE, prolonging pregnancy and improving outcomes. While C5 inhibition holds promise in treating pregnancy complications in APS, and complement split products are increased in non-pregnant patients with APS [72], plasma levels of complement C3 in pregnant patients with APS were not predictive of adverse pregnancy outcomes [73].

Not all complement components have detrimental effects on pregnancy—C1q is required for normal placentation

Despite the clear association between dysregulated complement activation and poor pregnancy outcomes, the presence of certain complement proteins is strictly required to assure normal placentation and pregnancy development. The important role of complement component C1q in normal placentation has been reported [74]. C1q is widely distributed in human decidual endothelial and stromal cells and is actively synthesized by migrating extravillous trophoblasts which require C1q for proper adhesion and migration [74]. Impaired placental labyrinth development, decidual vessel remodeling, and increased fetal death were observed in C1q-deficient mice [74, 75]. The contribution of C1q from fetal-derived trophoblasts in assuring good pregnancy outcome was demonstrated by mating C1q sufficient females with C1q-deficient males. C1q sufficient females showed increased fetal death and abnormal placentas only when impregnated by a male deficient in C1q, indicating that trophoblast-derived C1q is crucial for normal placentation and fetal survival. In addition, when compared to normal mice, C1q-deficient mice showed diminished decidual collagenase activity—required for trophoblast migration and placental angiogenesis—and thicker deciduas, providing additional evidence for defective trophoblast invasion of the maternal tissue in the absence of C1q [75]. It has been suggested that such defective invasion of trophoblasts into the maternal decidua is linked to PE. In line with this concept, pregnant C1q-deficient mice developed PE and recapitulated the key features of the human disease: hypertension, albuminuria, endotheliosis, endothelial dysfunction, decreased placental VEGF, and elevated levels of sFlt-1, previously correlated with increased rates of fetal death [75]. Increased sensitivity to angiotensin II and decreased vasorelaxant response to acetylcholine was observed in aortic rings isolated from pregnant C1q-deficient mice, indicating the presence of endothelial dysfunction in these mice. These studies highlight the crucial role of C1q in normal placentation and good pregnancy outcomes.

Conclusion

The hemiallogeneic fetus would be quickly rejected without numerous mechanisms that work in concert to protect the fetus from immunological recognition and rejection. The presence

of complement inhibitors at the maternal interface is an important mechanism that help pregnancy survival. Pregnancy complications such as recurrent miscarriages, preterm birth, intrauterine growth restriction, and preeclampsia have been associated with excessive complement activation in animal models and in humans, suggesting that complement split products might be used as biomarkers to predict pregnancy outcomes and blockage of the complement cascade might result in improved pregnancy outcomes. Randomized controlled trials should be organized to test this possibility in women.

References

1. Billingham RE, Brent L, Medawar PB (2010) Pillars article: "activity acquired tolerance" for foreign cells. J Immunol 184:5–8
2. Hanna J, Goldman-Wohl D, Hamani Y et al (2006) Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. Nat Med 12:1065–1074
3. Aagaard-Tillery KM, Siler R, Dalton J (2006) Immunology of normal pregnancy. Semin Fetal Neonatal Med 11:279–295
4. Fuzzy B, Rizzo R, Criscuoli L et al (2002) HLA-G expression in early embryos is a fundamental prerequisite for the obtainment of pregnancy. Eur J Immunol 32:311–315
5. Rouas-Freiss N, Marchal RE, Kirszenbaum M et al (1997) The alpha 1 domain of HLA-G1 and HLA-G2 inhibits cytotoxicity induced by natural killer cells : is HLA-G the public ligand for natural killer cell inhibitory receptors? PNAS 94:5249–5254
6. Munn DH, Zhou M, Attwood JT et al (1998) Prevention of allogeneic fetal rejection by tryptophan catabolism. Science 281:1191–1193
7. Trowsdale J, Betz AG (2006) Mother's little helpers : mechanisms of maternal-fetal tolerance. Nat Immunol 7:241–246
8. Moffett A, Colucci F (2014) Uterine NK-cells : active regulators at the maternal-fetal interface. J Clin Invest 124:1872–1879
9. Holmes CH, Simpson KL, Wainwright SD et al (1990) Preferential expression of the complement regulatory protein decay accelerating factor at the fetomaternal interface during human pregnancy. J Immunol 144:3099–3105
10. Hsi BL, Hunt JS, Atkinson JP (1991) Differential expression of complement regulatory proteins on subpopulations of human trophoblast cells. J Reprod Immunol 19:209–223
11. Janeway CA Jr, Travers P, Walport M et al (2001) Immunobiology: the immune system in health and disease, 5th edn. Garland Science, New York The complement system and innate immunity
12. Morgan BP, Meri S (1994) Membrane proteins that protect against complement lysis. Springer Semin Immunopathol 15:369–396
13. Youn-Uck K, Kinoshita T, Molina H et al (1995) Mouse complement regulatory protein Crry/p65 uses the specific mechanisms of both human decay-accelerating factor and membrane cofactor protein. J Exp Med 181:151–159
14. Xu C, Mao D, Holers VM et al (2000) A critical role for the murine complement regulator Crry in fetomaternal tolerance. Science 287: 498–501
15. Morgan BP, Holmes CH (2000) Immunology of reproduction: protecting the placenta. Curr Biol 10(10):R381–R383
16. Mao D, Wu X, Deppong C et al (2003) Negligible role of antibodies and C5 in pregnancy loss associated exclusively with C3-dependent mechanisms through complement alternative pathway. Immunity 19:813–822
17. Molina H, Wong W, Kinoshita T et al (1992) Distinct receptor and regulatory properties of recombinant mouse complement receptor 1 (CR1) and Crry, the two genetic homologues of human CR1. J Exp Med 175:121–129
18. Wu X, Spitzer D, Mao D et al (2008) Membrane protein Crry maintains homeostasis of the complement system. J Immunol 181(4):2732–2740
19. Lokki AI, Heikkinen-Eloranta J, Jarva H et al (2014) Complement activation and regulation in preeclamptic placenta. Front Immunol 5:312
20. Buurma A, Cohen D, Veraar K et al (2012) Preeclampsia is characterized by placental complement dysregulation. Hypertension 60: 1332–1337
21. Holmes CH, Simpson KL, Okada H et al (1992) Complement regulatory proteins at the feto-maternal interface during human placental development: distribution of CD59 by comparison with membrane cofactor protein (CD46) and decay accelerating factor (CD55). Eur J Immunol 22:1579–1585
22. Bulla R, Bossi F, Agostinis C et al (2009) Complement production by trophoblast cells at the feto-maternal interface. J Reprod Immunol 82:119–125
23. Mor G, Cardenas I, Abrahams V et al (2011) Inflammation and pregnancy: the role of the immune system at the implantation site. Ann N Y Acad Sci 1221:80–87
24. Baines MG, Millar KG, Mills P (1974) Studies of complement levels in normal human pregnancy. Obstet Gynecol 43:806–810
25. Hopkinson ND, Powell RJ (1992) Classical complement activation induced by pregnancy: implications for management of connective tissue diseases. J Clin Pathol 45:66–67
26. Richani K, Soto E, Romero R et al (2005) Normal pregnancy is characterized by systemic activation of the complement system. J Matern Fetal Neonatal Med 17:239–245
27. Risk JM, Flanagan BF, Johnson PM (1991) Polymorphism of the human CD46 gene in normal individuals and in recurrent spontaneous abortion. Hum Immunol 30:162–167
28. Mohlin FC, Mercier E, Fremeaux-Bacchi V (2013) Analysis of genes coding for CD46, CD55, and C4b-binding protein in patients with idiopathic, recurrent, spontaneous pregnancy loss. Eur J Immunol 43:1617–1629
29. Abdi-Shayan S, Monfaredan A, Moradi Z et al (2016) Association of CD46 IVS1-1724 C>G single nucleotide polymorphism in Iranian women with unexplained recurrent spontaneous abortion (URSA). Iran J Allergy Asthma Immunol 15:303–308
30. Salmon JE, Heuser C, Triebwasser M et al (2011) Mutations in complement regulatory proteins predispose to preeclampsia: a genetic analysis of the PROMISSE cohort. PLoS Med 8(3):e1001013
31. Lokki A, Aalto-Viljakainen T, Meri S et al (2015) Genetic analysis of membrane cofactor protein (CD46) of the complement system in women with and without preeclamptic pregnancies. PLoS One 10(2):–e0117840
32. WHO fact sheet. http://www.who.int/mediacentre/factsheets/fs363/en/
33. Gonzalez JM, Franzke CW, Yang F et al (2011) Complement activation triggers metalloproteinases release inducing cervical remodeling and preterm birth in mice. Am J Pathol 179:838–849
34. Norwitz ER, Caughey AB (2011) Progesterone supplementation and the prevention of preterm birth. Rev Obstet Gynecol 4:60–72
35. Fonseca EB, Celik E, Parra M et al (2007) Progesterone and the risk of preterm birth among women with a short cervix. N Engl J Med 357:462–469
36. Romero R, Nicolaides K, Conde-Agudelo A et al (2012) Vaginal progesterone in women with an asymptomatic sonographic short cervix in the midtrimester decreases preterm delivery and neonatal

morbidity: a systematic review and metaanalysis of individualpatient data. Am J Obstet Gynecol 206:e1–19
37. Norman JE, Marlow N, Messow CM et al (2016) Vaginal progesterone prophylaxis for preterm birth (the OPPTIMUM study): a multicentre, randomised, double-blind trial. Lancet 387:2106–2116
38. Gonzalez JM, Dong Z, Romero R et al (2011) Cervical remodeling/ripening at term and preterm labor: the same mechanism initiated by different mediators and different effector cells. PLoS One 6:e26877
39. Lynch AM, Gibbs RS, Murphy JR et al (2008) Complement activation fragment bb in early pregnancy and spontaneous preterm birth. Am J Obstet Gynecol 199:354.e1–354.e8
40. Lynch AM, Gibbs RS, Murphy JR et al (2011) Early elevations of the complement activation fragment C3a and adverse pregnancy outcomes. Obstet Gynecol 117:75–83
41. Lynch AM, Wagner BD, Deterding RR et al (2016) The relationship of circulating proteins in early pregnancy with preterm birth. Am J Obstet Gynecol 214:517.e1–517.e8
42. Volpe JJ (2009) Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. Lancet Neurol 8:110–124
43. Treyvaud K, Ure A, Doyle LW et al (2013) Psychiatric outcomes at age seven for very preterm children: rates and predictors. J Child Psychol Psychiatry 54:772–779
44. Rathbone R, Counsell SJ, Kapellou O et al (2011) Perinatal cortical growth and childhood neurocognitive abilities. Neurology 77: 1510–1517
45. Bell MJ, Hallenbeck JM (2002) Effects of intrauterine inflammation on developing rat brain. J Neurosci Res 70:570–579
46. Duncan JR, Cock ML, Scheerlinck JP et al (2002) White matter injury after repeated endotoxin exposure in the preterm ovine fetus. Pediatr Res 52:941–949
47. Pedroni SMA, Gonzalez JM, Wade J et al (2014) Complement inhibition and statins prevent fetal brain cortical abnormalities in a mouse model of preterm birth. Biochim Biophys Acta (BBA) - Mol Basis Dis 1842:107–115
48. Matute C (2011) Glutamate and ATP signalling in white matter pathology. J Anat 219:53–64
49. Girardi G, Fraser J, Lennen R et al (2015) Imaging of activated complement using ultrasmall superparamagnetic iron oxide particles (USPIO) - conjugated vectors: an in vivo in utero non-invasive method to predict placental insufficiency and abnormal fetal brain development. Mol Psychiatry 20:1017–1026
50. Pataky R, Howie FA, Girardi G et al (2016) Complement C5a is present in CSF of human newborns and is elevated in association with preterm birth. J Matern Fetal Neonatal Med 21:1–4
51. Chaouat G, Kiger N, Wegmann TG (1983) Vaccination against spontaneous abortion in mice. J Reprod Immunol 8:389–394
52. Girardi G, Yarilin D, Thurman JM et al (2006) Complement activation induced dysregulation of angiogenic factors and causes fetal rejection and growth restriction. J Exp Med 203:2165–75
53. Ahmed A, Singh J, Khan Y et al (2010) A new mouse model to explore therapies for preeclampsia. PLoS One 27 5(10):e13663
54. Petitbarat M, Durigutto P, Macor P et al (2015) Critical role and therapeutic control of the lectin pathway of complement activation in an abortion-prone mouse mating. J Immunol 195:5602–5607
55. Qing X, Redecha PB, Burmeister MA et al (2011) Targeted inhibition of complement activation prevents features of preeclampsia in mice. Kidney Int 79:331–339
56. Xia Y, Kellems RE (2013) Angiotensin receptor agonistic autoantibodies and hypertension: preeclampsia and beyond. Circ Res 113: 78–87
57. Kim EN, Yoon BH, Lee JY et al (2015) Placental C4d deposition is a feature of defective placentation: observations in cases of preeclampsia and miscarriage. Virchows Arch 466:717–725
58. Derzsy Z, Prohaszka Z, Rigo J et al (2010) Activation of the complement system in normal pregnancy and preeclampsia. Mol Immunol 47:1500–1506
59. Soto E, Romero R, Richani K et al (2010) Preeclampsia and pregnancies with small-for-gestational age neonates have different profiles of complement split products. J Matern Fetal Neonatal Med 23:646–657
60. Burwick RM, Fichorova RN, Dawood HY et al (2013) Urinary excretion of c5b-9 in severe preeclampsia: tipping the balance of complement activation in pregnancy. Hypertension 62:1040–1045
61. Tichenor JR, Bledsoe LB, Opsahl MS et al (1995) Activation of complement in humans with a first trimester pregnancy loss. Gynecol Obstet Investig 39:79–82
62. Salmon JE, Girardi G (2007) Theodore E. Woodward Award : Antiphospholipid Syndrome Revisited : a disorder initiated by inflammation. Trans Am Clin Climatol Assoc 118:99–114
63. Girardi G, Berman J, Redecha P et al (2003) Complement C5a receptors and neutrophils mediate fetal injury in the antiphospholipid syndrome. J Clin Invest 112:1644–1654
64. Peerschke EIB, Yin W, Alpert DR et al (2009) Serum complement activation on heterologous platelets is associated with arterial thrombosis in patients with systemic lupus erythematosus and antiphospholipid antibodies. Lupus 18:530–538
65. Redecha P, Tilley R, Tencati M et al (2007) Tissue factor link between C5a and neutrophil activation in antiphospholipid antibody induced fetal injury. Blood 110:2423–2431
66. Redecha P, Franzke CW, Ruf W et al (2008) Neutrophil activation by the tissue factor/factor VIIa/PAR2 axis mediates fetal death in a mouse model of antiphospholipid syndrome. J Clin Invest 118: 3453–3461
67. http://www.thescientist.com/?articles.view/articleNo/27610/title/Safeguardingthe-Foreigner-Within/
68. Bertolaccini ML, Contento G, Lennen R et al (2016) Complement inhibition by hydroxychloroquine prevents placental and fetal brain abnormalities in antiphospholipid syndrome. J Autoimmun 75:30–38
69. Mahajan SD, Parikh NU, Woodruff TM et al (2015) C5a alters blood-brain barrier integrity in a human in vitro model of systemic lupus erythematosus. Immunology 146:130–143
70. Gustavsen A, Skattum L, Bergseth G et al (2017) Effect on mother and child of eculizumab given before caesarian section in a patient with severe antiphospholipid syndrome : a case report. Medicine (Baltimore) 96(11):e6338
71. Burwick RM, Feinberg BB (2013) Eculizumab for the treatment of preeclampsia/HELLP syndrome. Placenta 34:201
72. Breen KA, Seed P, Parmar K et al (2012) Complement activation in patients with isolated antiphospholipid antibodies or primary antiphospholipid syndrome. Thromb Haemost 107:423-429
73. Buyon JP, Kim MY, Guerra MM et al (2015) Predictors of pregnancy outcomes in patients with lupus: a cohort study. Ann Intern Med 163:153-163
74. Agostinis C, Bulla R, Tripodo C et al (2010) An alternative role of C1q in cell migration and tissue remodeling: contribution to trophoblast invasion and placental development. J Immunol 185:4420-4429
75. Singh J, Ahmed A, Girardi G (2011) Role of complement component C1q in the onset of preeclampsia in mice. Hypertension 58: 716-724