# Regulators of Complement System Activation Increase with Placental Ischemia-induced Hypertension in Rat

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Abstract 628

Strehlke ME, Elmquist BJ, Bemis LT, Gilbert JS, Regal JF
Department of Biomedical Sciences, University of Minnesota Medical School Duluth, Duluth, Minnesota

## Abstract

Preeclampsia is characterized by new onset hypertension, reduced placental perfusion, and increased activation of complement, part of the innate immune system. In a rat model of placental ischemia-induced hypertension, our previous work was the first to demonstrate the importance of complement activation, particularly activation products C3a and C5a, in mediating maternal hypertension. In this model circulating C3a increases, suggesting either increased activation of complement through C3 and/or insufficient changes in endogenous regulators to limit complement activation. In preeclampsia, complement regulators CD55 and CD59 have been shown to increase in placenta compared to normal pregnancy, indicating upregulation to control excessive complement activation. In addition, excessive complement activation in kidney has been demonstrated in preeclampsia. **Thus, we hypothesized that increased complement activation following placental ischemia in rat leads to an increase in complement regulators in kidney and placenta.** On gestation day (GD) 14, rats underwent either Sham surgery or clip placement on ovarian arteries and abdominal aorta to reduce uterine perfusion pressure (RUPP) resulting in increased maternal blood pressure on GD19. Serum C3a and soluble C5b-9 were measured as indicators of complement activation using Western Blot and ELISA assay, respectively. Membrane bound complement regulators investigated included: 1) CD55 and Crry which limit complement activation through C3 and C5, and 2) CD59 which limits formation of C5b-9 membrane attack complex. Kidney cortex and placenta were collected, mRNA extracted, and quantitative RTPCR used to measure mRNA for complement regulators CD55, CD59, and Crry using β-actin as a housekeeping gene. As expected, MAP significantly increased ($p<0.05$) in RUPP (109±3 mmHg, n=9) vs. Sham (95±3 mmHg, n=7) animals, with a corresponding increase in C3a (0.49±0.14 units/ul, RUPP; 0.22±0.09 units/ul, Sham). Soluble C5b-9 did not significantly differ in plasma of RUPP vs. Sham animals. In placenta, mRNA for CD55, CD59, or Crry did not significantly change with placental ischemia. However, in kidney cortex CD59 mRNA significantly increased in RUPP vs. Sham with a 1.16±0.04 fold change ($p<0.05$). A slight increase in CD55 mRNA was noted (1.21±0.09 fold change; p= 0.07) in RUPP vs. Sham kidney cortex, and no change in Crry mRNA was detected.Thus, our data demonstrate increased CD59 mRNA in kidney following placental ischemia with a minor increase in CD55, without upregulation of either molecule in placenta. The minor increase in CD55 in kidney and lack of change in Crry in either placenta or kidney following placental ischemia suggests inadequate regulation at the level of C3 and C5 activation resulting in increased C3a. However, increased CD59 in kidney following placental ischemia limits production of soluble C5b9.Thus, placental ischemia alone in the rat does not change complement regulators in the placenta. However, these data suggest a feedback mechanism is present in kidney that adequately limits circulating C5b-9 but is insufficient to limit excessive C3a production.

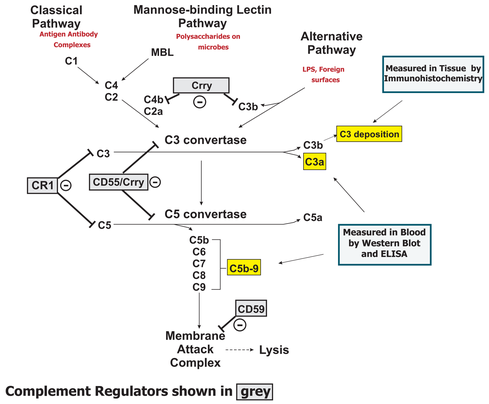
## Background & Rationale

**Preeclampsia** is a pregnancy specific condition characterized by:
- New onset hypertension and often proteinuria
- Abnormal placental development with reduced placental perfusion and ischemia

### Complement System and Preeclampsia
- The complement system is part of the innate immune system
- Complement activation is greater in blood, placenta, and kidney in preeclamptic pregnancies compared to normal pregnancies (Buurma et al, 2012; Penning et al, 2015)
- In rat, our previous studies demonstrated that preventing complement activation attenuates placental ischemia-induced hypertension (Lillegard et al, 2013)

### Complement System Regulators
- Complement regulators are endogenous membrane proteins that limit complement activation on 'self'
- In preeclampsia, an increase in complement regulators in placenta has been shown in human pregnancy compared to normal pregnancy (Buurma et al, 2012)
- The net complement activation is determined by both the degree of activation of the pathway and the expression levels of endogenous regulators that dampen the activation

**Net complement activation** = **Activation of pathway by stimuli** − **Dampening by endogenous regulators (Crry, CD55, CD59)**




**Complement Regulators shown in grey**

### Crry
- Complement receptor 1-related gene protein Y
- Specific to mouse and rat
- Functions similarly to CD55 and CD46 in human

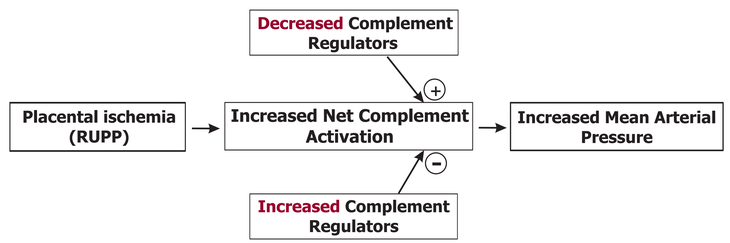
### CD55
- Limits complement activation through dissociation of C3 and C5 convertase

### CR1
- Limits complement activation through inhibition of C3 and C5 convertase

### CD59
- Controls formation of the C5b-9 membrane attack complex

## Hypothesis

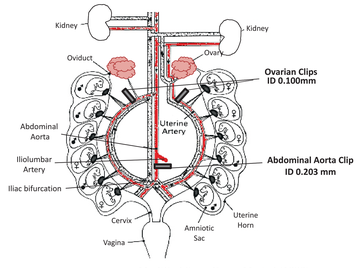
**Increased complement activation following placental ischemia in rat leads to an increase in complement regulators in kidney and placenta.**




## Model of Placental Ischemia

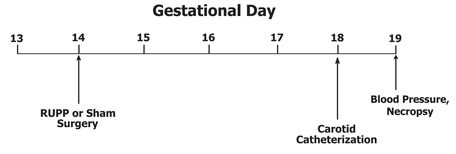
### Reduced uterine perfusion pressure (RUPP) model of placental ischemia-induced hypertension

On gestation day (GD) 14 (Sprague Dawley rats, Charles River), silver clips are placed on the lower abdominal aorta and the ovarian arteries to decrease blood flow to the placenta and increase maternal blood pressure on GD19.




Modified from Even, M et al (1992)

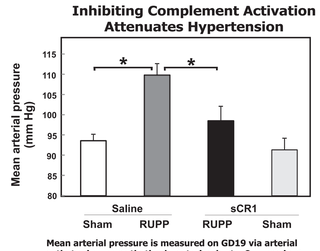
## Experimental Design




## Results

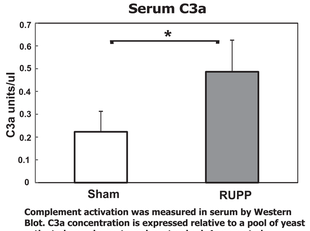
### [Previous Studies]

**Inhibiting Complement Activation Attenuates Hypertension**



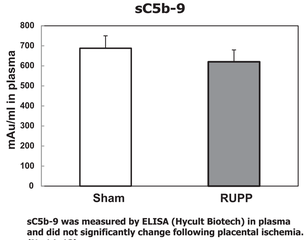

**Mean arterial pressure is measured on GD19 via arterial catheter in unanesthetized, restrained rats. Our previous studies demonstrated that administering complement inhibitor sCR1 attenuates placental ischemia-induced hypertension (Lillegard et al, 2013). (N=22, 19, 9, 6; *p<0.05)**

**Serum C3a**



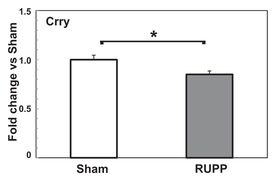

**Complement activation was measured in serum by Western Blot. C3a concentration is expressed relative to a pool of yeast activated complement used as standard. As expected, complement activation was increased following placental ischemia. (N =7, 10; *p<0.05)**

**sC5b-9**



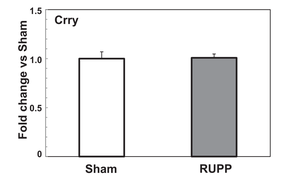

**sC5b-9 was measured by ELISA (Hycult Biotech) in plasma and did not significantly change following placental ischemia. (N=14, 13)**

**Placental ischemia decreased Crry and CD59 message in placenta**



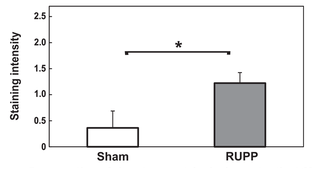

**RNA was extracted from frozen placenta and message for Crry, CD55, and CD59 measured using quantitative RT-PCR with β-actin as a housekeeping gene. (N=4, 6; *p<0.05)**

**Placental ischemia increased CD55 and CD59 message in kidney**



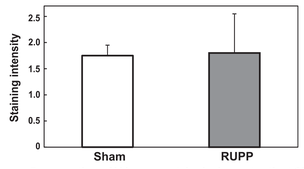

**RNA was extracted from frozen kidney cortex and message for Crry, CD55, and CD59 measured using quantitative RT-PCR with β-actin as a housekeeping gene. (N=8, 7; *p<0.05)**

**Placental ischemia increased C3 deposition in placenta**




**Frozen sections of placenta were stained using goat anti-rat C3 (MP Biochem, 55713), followed by fluorescent secondary antibody Alexa Fluor 594 donkey anti-goat (Jackson Immunoresearch). Staining was graded by a blinded observer. (N=9, 11; *p<0.5)**

**Placental ischemia does not change C3 deposition in kidney**




**Frozen sections of kidney were stained using goat anti-rat C3 (MP Biochem, 55713), followed by fluorescent secondary antibody Alexa Fluor 594 donkey anti-goat (Jackson Immunoresearch). Staining was graded by a blinded observer. (N=4, 5; *p<0.5)**

## Conclusions

**Placental ischemia-induced changes in complement regulators are dependent on the tissue type**

**Placenta**

**In placenta, the data suggest that the decrease in endogenous regulators contribute to the net increase in complement activation as demonstrated by increased C3 deposition and circulating C3a**

**Kidney**

**In kidney, the increase in complement regulators is sufficient to limit C3 deposition, suggesting that complement activation in kidney does not contribute to increased circulating C3a following placental ischemia**

**References:**

Buurma A, Cohen D, Kimberley Veraar, Schonkeren D, Claas FH, Bruijn JA, Bloemenkamp KW & Baelde HJ. (2012) Hypertension, 60, 1332-1337.
Even, M. et al; J. Reprod. Fert. (1992) 96, 709-716.
Lillegard KE, Johnson AC, Lojovich SJ, Bauer AJ, Marsh HC, Gilbert JS, Regal JF (2013) Molecular Immunology, 56, 91-97
Penning M, Chua JS, Kooten C, Zandbergen M, Buurma A, Schutte J, Bruijn JA, Khankin EV, Bloemenkamp K, Karumanchi SA & Baelde H. (2015) Hypertension, 66, 00-00)

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**Email: streh036@d.umn.edu**