ONLINE SUPPLEMENT TO

DIFFERENTIAL EFFECTS OF COMPLEMENT ACTIVATION PRODUCTS C3A AND C5A ON CARDIOVASCULAR FUNCTION IN HYPERTENSIVE PREGNANT RATS

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Running title: C3a, C5a and pregnancy-induced hypertension

Reduced utero-placental perfusion pressure (RUPP) procedure

In brief, surgical procedures were performed with timed pregnant Sprague Dawley dams (Crl:CD IGS, Charles River Laboratories, Portage, MI) under isoflurane anesthesia. On gestation day 14 of a 21 day gestation (Day 0 defined as breeding date), rats weighed 292 ± 2 g (n=85). Sterile silver clips of 0.203 mm inner diameter were placed around the aorta above the iliac bifurcation. In addition, silver clips of 0.100 mm inner diameter were placed on both right and left uterine arcades at the ovarian end, right before the first segmental artery to prevent the adaptive increase in ovarian blood flow. The jugular vein was catheterized for administration of antagonists. Since heparin is known to affect complement activation, a 25% dextrose lock solution was used to maintain catheter patency. All control animals underwent Sham surgery differing only in the absence of clip placement. Specific individual contrasts evaluated statistically and presented in figures were: 1). Sham Veh vs RUPP Veh, 2). RUPP vs RUPP C3aRA, 3). RUPP vs RUPP C5aRA, 4). Sham vs Sham C3aRA, 5). Sham vs Sham C5aRA.

Measurement of neutrophils in the lung

To determine if the decreased number of circulating neutrophils was due to their sequestration in the lung, lung lobes were removed on GD19 and processed as previously described for determination of myeloperoxidase content as an indicator of the number of neutrophils in the tissue at necropsy (Fraser et al., 1995). Protein content was determined using the DC Protein assay method (BioRad, Hercules, CA). Myeloperoxidase was expressed as Units MPO activity per mg protein in supernatants of detergent extracted cell homogenate.

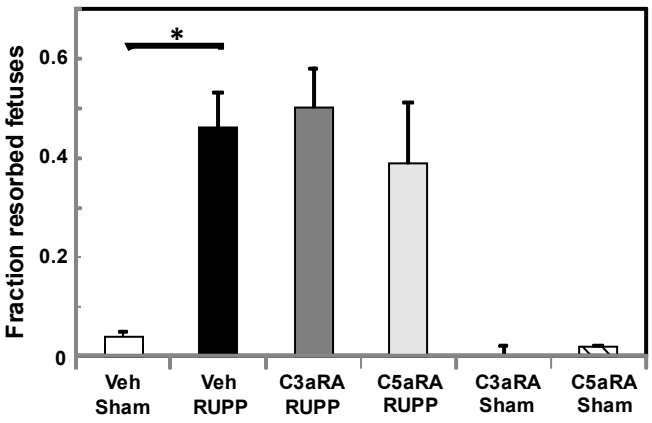

Figure S1: C3a and C5a receptor antagonists do not attenuate fetal resorptions following placental ischemia. Sham or RUPP animals were treated with vehicle (Veh), C3a receptor antagonist (C3aRA) or C5a receptor antagonist (C5aRA) from GD14-18. Values represent mean ± SE of fraction of fetuses resorbed measured on GD19. *$p<0.05$ for indicated comparisons. The fraction of fetuses resorbed increased in RUPP Veh (n=23) compared to Sham Veh (n=19), and was not significantly altered by C3aRA (n=12) or C5aRA (n=11). The fraction resorbed did not differ between Sham animals treated with Veh, C3aRA (n=6) or C5aRA (n=5).

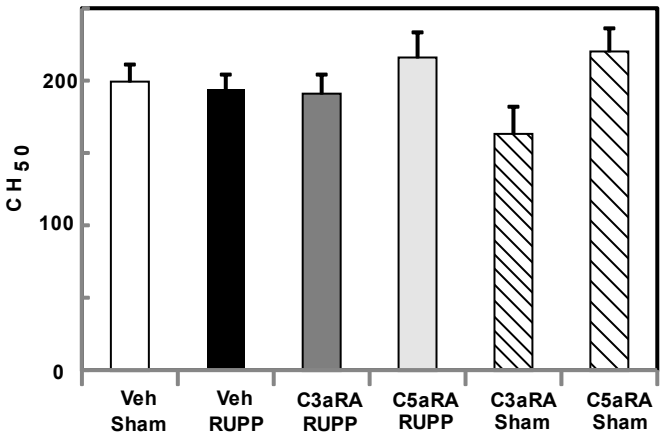

Figure S2: Total hemolytic complement activity (CH_{50}) in serum does not differ amongst treatment groups. Sham or RUPP animals were treated with vehicle (Veh), C3a receptor antagonist (C3aRA) or C5a receptor antagonist (C5aRA) from GD14-18. The CH_{50} in RUPP Veh (n=23) did not differ from ShamVeh (n=19), RUPP C3aRA (n=12) or RUPP C5aRA (n=11) treated animals. CH_{50} also did not differ between Sham animals treated with Veh (n=19), C3aRA (n=6) or C5aRA (n=5). Values represent geometric mean ± SE of CH_{50} measured from serum collected on GD19. *$p<0.05$ for indicated comparisons.

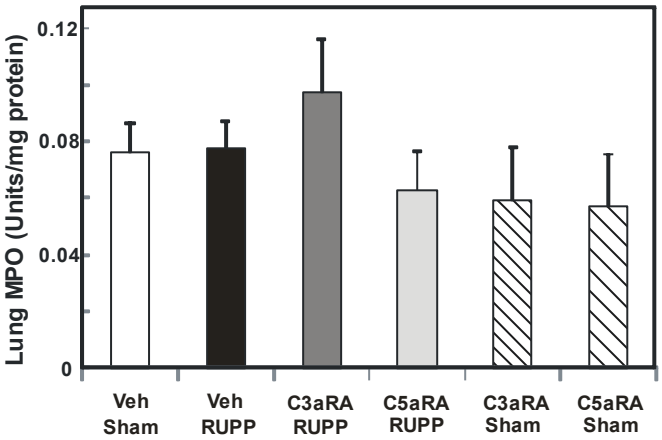

Figure S3: Effect of placental ischemia and antagonists on neutrophil sequestration in lung. Sham or RUPP animals were treated with vehicle (Veh), C3a receptor antagonist (C3aRA) or C5a receptor antagonist (C5aRA) from GD14-18. Treatment with either the C3aRA or the C5aRA did not alter neutrophils in the lung. Values represent mean ± SE of lung MPO/mg protein. Lung MPO was measured in homogenized lung tissue from GD19 to estimate the number of neutrophils in the tissue as described in supplementary methods.

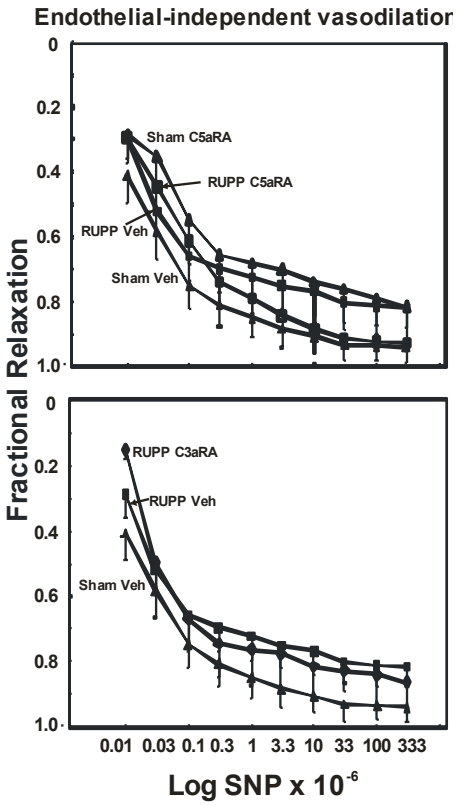

Figure S4: Neither C5a receptor antagonist (C5aRA) or C3a receptor antagonist (C3aRA) prevents placental ischemia-induced endothelial independent vasodilation. Sham or RUPP animals were treated with vehicle (Veh), C3aRA or C5aRA from GD14-18. Mesenteric arteries were isolated on GD19, pre-contracted with the thromboxane mimetic U46619 and the relaxation to sodium nitroprusside (SNP; endothelial-independent vasodilation) assessed. Relaxation to SNP was not altered in RUPP Veh (n=14) compared to Sham Veh (n=10). Neither the C3aRA nor the C5aRA altered SNP vasodilation. Values represent mean ± SE of fractional relaxation in pre-contracted arteries. No differences were detected.

Fraser DG, Regal JF and Arndt ML (1995) Trimellitic anhydride-induced allergic response in the lung: role of the complement system in cellular changes. *J Pharmacol Exp Ther* **273**:793-801.