Preeclampsia Foundation Mid-Year Progress Report

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We would like to thank the Preeclampsia Foundation for giving us the opportunity to investigate the contribution of placenta-derived factors to the development of preeclampsia.

Project Goal: The goal of this project is to test the hypothesis that molecules released from the placenta trigger receptors of the immune system in the blood vessels of pregnant women that can lead to global inflammation. We believe that this inflammatory response impairs the normal development of the growing fetus because vessels supplying the fetus with oxygen and nutrients become dysfunctional. Further, we suggest that global inflammation leads to elevation of maternal blood pressure because the maternal vascular system does not function properly. Collectively, we propose that the activation of the maternal immune system by placenta-derived molecules causes maternal hypertension and intrauterine growth restriction.

Study Objectives:

1. To examine the molecular mechanisms, by which activation of immune receptors during pregnancy causes inflammation, elevated levels of protein in maternal urine and maternal hypertension.
2. To examine the molecular mechanisms by which activation of immune receptors cause dysfunction of the maternal vessels.
3. To measure mitochondrial DNA in the circulation of pregnant women with and without preeclampsia and to determine whether there is an association between these measures and inflammation.

To address objectives 1 and 2, we proposed to use an experimental animal model and to address objective 3 we would use blood samples from pregnant women.

Progress (January 2013 – July 2013)
During this period, we have performed experiments to address objectives 1 and 2. Results from these experiments have been presented in two annual national conferences: Experimental Biology, April 20-24, 2013, Boston, Massachusetts and Society for Gynecologic Investigation, March 20-23, 2013, Orlando, Florida. These results are described below:

1. We propose that the molecules that trigger the maternal immunes system in preeclampsia are mitochondrial DNA that are released in the circulation from damage placental cells. In order to mimic the effects of mitochondrial DNA on immune receptors, we used a pharmacological approach and treated non-pregnant and pregnant rats with a drug (ODN 2395) that activates the same receptors with those activated by mitochondrial DNA. Rat pregnancy lasts approximately 22 days. On gestational day 20 (late pregnancy), we measured blood pressure in treated and untreated pregnant and non-pregnant rats. On gestational day 21, we tested the reactivity of maternal vessels and stored vascular tissues for subsequent biochemical analysis to measure inflammatory markers. The treatment with ODN 2395 increased blood pressure in pregnant but not in non-pregnant rats. In addition, vascular responsiveness to compounds that induce constriction was greater in vessels from pregnant rats treated with ODN 2395. Fetuses from rats treated with ODN 2395 were smaller compared to untreated rats. Biochemical analysis in vascular tissues revealed that ODN 2395 treatment results in increase of inflammatory markers in maternal vessels. Inflammation also increases in vessels from non-pregnant rats, although their blood pressure does not change. **Conclusion:** Activation of the immune system by a drug that activates the same receptor with that activated by mitochondrial DNA causes maternal hypertension, enhanced constriction of the vessels, reduction of fetal growth, maternal hypertension and vascular inflammation. These results support our hypothesis that circulating

placenta-derived mitochondrial DNA induces preeclampsia-like symptoms via activation of the immune system in rats. **Future directions:** We are currently performing experiments to examine the exact molecules that are involved in the activation of the immune system in this animal model (treated with ODN 2395) and we are planning to treat rats with mitochondrial DNA derived from rat placentas to test whether they develop preeclampsia-like symptoms.

2. In our preliminary studies, we had found that treatment of pregnant rats with damaged rat mitochondria causes maternal hypertension. We have now repeated these studies. We isolated mitochondria from rat liver and we purposely caused their damage (damaged mitochondria). Then, we injected them in pregnant rats at mid-pregnancy and after 3 days, we sacrificed the animals and collected their placentas for biochemical analysis. Our results indicate a significant increase in the amount of proteins that are associated with cell death and inflammation in rat placentas. Inflammation and exaggerated placental cell death are features of pregnancies with preeclampsia. **Conclusion:** In addition to maternal hypertension, circulating damaged mitochondria cause inflammation and increased rates of cell death in placentas from rats. **Future directions:** Damaged mitochondria include a lot of proteins as well as mitochondrial DNA. Therefore, our results cannot be attributed to a specific molecule. Accordingly, we would like to perform experiments, in which we will identify which exactly mitochondrial molecules act in synergy with mitochondrial DNA causing maternal hypertension and attenuating fetal growth.