Status Report for Preeclampsia Foundation Vision Grant 2010

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PROJECT TITLE Maternal-fetal immune tolerance in preeclampsia

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Our approach to the scope of work encompassed by the grant has been to divide the work into two phases: first, a subject recruitment/assay optimization phase, and second, a phase of completion of the experiments. To this end, we have focused on the first phase during the first half of the grant and plan to complete the second phase during the latter half.

Subject Recruitment:
For Specific Aim 1, we are seeking to evaluate the balance between regulatory T cell (T_{reg}) and T_{H}17 cell subsets in women with preeclampsia compared with gestational age-matched controls. Our power analysis suggested the need for 25 preeclampsia cases and 25 gestational-age matched controls. (The estimated number of subjects needed for Aim 2 is substantially lower, approximately 10 in each group.) In order to maximize the uniformity of the preeclampsia group, we have chosen to limit the group to primigravid women without underlying hypertension, meeting criteria for severe preeclampsia. A precise range of inclusion criteria is ideal in an investigation of immunologic contributions to preeclampsia because it is among this population of women that immune dysfunction is most likely to be important.

To facilitate this, we have now enrolled and stored paired maternal/cord blood samples for 45 subjects without a history of underlying hypertension who met criteria for severe preeclampsia. Of these, 34 had had no prior deliveries (parity=0), and 21 were primigravid (gravidity=1 and parity=0). This resource of samples provides us with adequate power to address all of the proposed grant aims.

Assay Optimization:
Several experimental approaches required optimization before the subject samples could be tested. These included: cytokine assays, flow cytometry and fluorescence-activated cell sorting (FACS) techniques, intracellular staining, cell culture, and assays to measure cellular proliferation in the functional studies (primarily carboxyfluorescein diacetate succinimidyl ester (CFSE) staining as measured by flow cytometry). We have optimized all of these approaches and have specifically delineated the laboratory protocols for the experimental approach that will be taken for each subject.

The three most complex assays that required optimization were the technique use to quantify and deplete maternal T regs, the intracellular staining, and the CFSE detection after maternal-fetal combined cell culture. These are the techniques used to enumerate maternal T regs, to determine if the T regs are responsible for quieting the maternal anti-fetal immune response, and to measure this immune response. Representative data from each of these techniques follow.

For the T reg depletion assay, we had originally planned to use magnetic bead assisted cell sorting (MACS) techniques. When working to optimize this assay, we found that its specificity was inadequate – while it did allow for T reg depletion, it also removed many effector cells, leading to imprecise results. We chose to instead use FACS techniques to specifically remove only T reg cells, and we have optimized this technique.
This figure shows a representative FACS plot:

![FACS plot](PEP_036A_TREGs.fcs)

T regs are defined as CD45+/CD3+/CD4+/CD25hi/CD127lo. This technique yields a frequency of T reg and allows for removal of this specific population of cells prior to maternal/fetal co-culture. The removed T reg cells are then evaluated for their purity by intracellular staining for Foxp3.

This figure shows a representative Foxp3 plot for the T reg population:

![Foxp3 plot](PEP_036A_CD25hi_CD127lo_foxp3_good.fcs)

Maternal immune reactivity against fetal cells is then measured after co-culture of maternal and fetal cells. We evaluated two techniques that measure cell proliferation (³²P incorporation and CFSE staining) to determine the best output measure for this, and we found that the CFSE detection assay yielded the best results.
This figure shows a representative CFSE plot after cell culture conditions were optimized:

This method allows for quantitative comparison of T cell proliferation as a measure of the maternal anti-fetal immune response across samples in a consistent, reproducible manner.

**Conclusions:**
Our goal with this work is to test the hypothesis that inadequate immunologic tolerance of the fetus by the mother contributes to the development of preeclampsia. We specifically aim to evaluate the balance between T reg and Th-17 cell populations in preeclampsia and to assess the functional capacity of T reg to suppress the maternal anti-fetal response in preeclampsia. In this first phase of this work, we have accumulated a sufficient resource of samples to complete the planned experiments with adequate statistical power to definitively address the aims, using stringent inclusion criteria that will maximize discovery. We have also worked over the past several months to systematically delineate the optimal assay conditions and approaches and to create a specific protocol which will now be utilized to answer these questions.

We are grateful to the Preeclampsia Foundation for the opportunity to explore immunologic contributions to a disease with such severe maternal and fetal consequences, and we are hopeful that our results will inform future directions for investigation and possible novel therapeutic targets.