Progress Report for Alexander Panda M.D., M.P.H.

Since June 2009 we have collected blood from **30 women with severe preeclampsia**. We have analyzed dendritic cell samples for controls and from women with severe preeclampsia using flow cytometry.

Dendritic Cell Isolation: PBMC from peripheral blood were collected in sodium-heparin tubes using the Histopaque gradient method. These PBMC were then stained with fluorescent labeled antibodies directed against dendritic cell markers using the following antibodies: CD123-PE (anti-interleukin-3 receptor), CD11c-APC, HLA-DR-PerCP, Lin-1 FITC (CD3, CD14, CD16, CD19, CD20 and CD56) as well as anti-TLR antibodies for TLR1, TLR2, TLR4, and TLR6, TLR7, TLR8 and TLR9 or matching isotype control antibodies. Cells were fixed and frozen at -80º C until analysis by flow cytometry.

Dendritic Cell TLR function: To assess TLR function, PBMC were stimulated with the following specific TLR ligands: Pam3CSK4 (TLR1/2 dimer), LTA (TLR2/6 dimer), LPS (TLR4), flagellin (TLR5), Gardiquimod TLR7, CL075 (TLR8) and CPG (TLR9). Brefeldin A, a membrane transport inhibitor, was added for the last hours of incubation to ensure intracellular accumulation of cytokines. These cells were then stained with dendritic cell markers using the following antibodies: CD123-PE (anti-interleukin-3 receptor), CD11c-APC, HLA-DR-PerCP, Lin-1 FITC (CD3, CD14, CD16, CD19, CD20 and CD56). After surface staining cells were fixed and frozen at -80º C until analysis by flow cytometry.

We evaluated TLR function in DCs from 30 controls and 30 women with severe preeclampsia. Notably, the group of women with severe preeclampsia represents a generally healthy group, with nearly 90% reporting no comorbid illnesses. No heterogeneity was found in terms of race, and number of comorbid illnesses. We used mixed effects multivariable statistical modeling to adjust for these covariates as in our previous work.

To elucidate the consequences of preeclampsia on TLR function in primary human dendritic cells, we employed multicolor flow cytometry and intracellular cytokine staining in peripheral blood mononuclear cell (PBMC) samples from controls and women with severe preeclampsia. Our results appear very promising. We are currently analyzing our data and expect our data to be submitted for publication by mid 2010 at which point we will post a full report on the Preeclampsia foundation's webpage.

Money Spent:
Approximately 22,000 USD have been spent for antibodies, consumables, patient compensation and software. We expect that the remainder of the budget will be used until June 2010.