Ex vivo Interferon Gamma Release Assay Responses to Cryptococcal Capsule Antigen Predict Outcomes of Death or Immune Reconstitution Inflammatory Syndrome after Cryptococcal Meningitis

Darin Wiesner[1], A Akamps[2], J Rhein[1,3], D Williams[1,3], D Meya[1,2,3], P Bohjanen[1], K Nielsen[1], D Boulware[1], and Cryptococcal Optimal ART Timing Trial
[1]Univ of Minnesota, Minneapolis, US; [2]Makerere Univ, Kampala, Uganda; and [3]Infectious Disease Inst, Kampala, Uganda

Background: Cryptococcal meningitis (CM) affects more than 1 million people with AIDS worldwide and causes 20-25% of AIDS-related mortality. We evaluated *ex vivo* cytokine responses to cryptococcal antigens in whole blood from persons with CM to determine if cytokine responses correlated with clinical outcomes such as immune reconstitution inflammatory syndrome (IRIS) or death.

Methods: 66 HIV^+, ART-naïve subjects with AIDS and acute CM were enrolled at Mulago Hospital in Kampala, Uganda, from November 2010 to June 2012. Whole blood from these subjects was diluted 2-fold with sterile phosphate buffered saline (PBS) and stimulated with PBS (negative control), purified cryptococcal capsule polysaccharide glucuronoxylomannan (GXM), purified cryptococcal mannoprotein, or crude cell wall extract (containing a mixture of GXM and mannoprotein) followed by culture for 16-20 hours at 37°C and 5% CO_2. The culture was separated by centrifugation and supernatants were stored at –80°C. Cytokine levels were analyzed using Luminex 17-plex assays (Bio-Rad). The net secreted cytokine levels were compared by group with t test with adjustment for multiple comparisons and then multivariate logistic regression. Quantitative CSF fungal cultures were also performed.

Results: In comparing subjects who died or developed CM-IRIS (n = 36) and subjects who recovered from CM (n = 30), after GXM-stimulation increased levels of interferon-γ (IFN-γ) ($p = 0.1$) and tumor necrosis factor-a (TNF-α) ($p = 0.07$) and lower levels of GM-CSF ($p = 0.1$) were associated with death or IRIS in a univariate analysis. In a multivariate analysis, increased cerebrospinal fluid (CSF) fungal burden assessed by quantitative culture (odds ratio [OR] = 2.4 per $\log_{10}$ colony-forming units [CFU]/mL, $p = 0.004$), increased GM-CSF levels (OR = 1.19 per 2-fold increase, $p = 0.014$), and decreased IFN-γ levels (OR = 0.806 per 2-fold increase, $p = 0.014$) in response to GXM antigen were associated with elevated risk of death or IRIS. The model correctly classified cases and controls with C-statistic = 0.76 ($p < 0.001$). Using an identical analysis, cytokine levels following stimulation of whole blood with the crude cell wall, or purified mannoprotein preparations did not correlate with CM outcomes.

Conclusions: Decreased *ex vivo* IFN-γ secretion in response to stimulation with GXM antigen was associated with increased risk of death or IRIS. Measuring *ex vivo* cytokine responses in whole blood could be used to identify patients who are unable to produce adequate amounts of IFN-γ and perhaps identify patients who could benefit from treatment with exogenous IFN-γ.