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P2308 Are you TUF enough? Enhanced expression of Elongation Factor Tu (TUF) from *Streptococcus pneumoniae* in the cerebrospinal fluid of adults with bacterial meningitis may cause worse clinical outcomes by enhancing pneumolysin-mediated neutrophil lysis

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Background:

Mortality from bacterial meningitis, predominately caused by *Streptococcus pneumoniae*, is persistently high in Low and Middle Income Countries in sub-Saharan Africa with high HIV prevalence. Low CSF leukocyte counts are a strong predictor of poor outcome, but the underlying mechanisms are poorly understood. We examined if the CSF proteome differed between adult survivors and non-survivors of pneumococcal meningitis (PM) in Malawi, and tested if transcription of differentially expressed proteins correlated with neutrophil activity.

Materials/methods:

CSF proteomes from adults with proven PM were analysed by quantitative Mass-Spectrometry. Spectra were identified using the Swissprot human and TIGR4 pneumococcal protein databases. Proteins with Uniprot identification were quantitated and analysed against clinical outcome. Host and pathogen transcriptional profiling of ribo-depleted total RNA was done in CSF of adults with proven PM by RNAseq, correlations between gene expression done by Spearman's-rank. Gene knock-out pneumococcal mutants were made in TIGR4 *S.pneumoniae* and tested for susceptibility to neutrophil mediated killing *in-vitro*.

Results:

CSF proteomes were available for 57 Adults with PM (median age 32 years, 60% male, 70% HIV-1 co-infected, mortality 63%). 360 individual human and 23 pneumococcal proteins were identified in the CSF. Of the human protein hits, 30% were not expressed in normal CSF and were primarily related to neutrophil activity. No human protein was associated with outcome. However, expression of the bacterial protein *S. pneumoniae* Elongation Factor Tu (*TUF*) was significantly increased in CSF of non-survivors (False Discovery Rate (q) <0.001). Expression of *TUF* in the CSF transcriptome of 11 patients with PM was correlated against human transcripts. *TUF* was highly co-correlated with 9 genes (R^2 >0.9 p<0.001), including the neutrophil expressed *HLA-A* and *CDHR-2*, an endothelial cadherin associated with neutrophil trans-migration and neutrophil-mediated inflammation.

A TIGR4 *TUF* knockout strain (Δ*TUF*) was constructed using overlap extension PCR and CSP-stimulated transformation. Data on the specific interaction between *TUF* , pneumolysin expression and neutrophil mediated killing will be presented (work in progress).

Conclusions:

Expression of *TUF* in CSF is implicated in poor clinical outcomes from pneumococcal meningitis. Attenuation of

TUF function *in vivo* may enhance neutrophil-mediated killing, and presents a potential target for adjunctive therapy.