Exploring Natural Killer (NK) Cell Responses in Typhoid Vaccination Using a Re-stimulation Assay

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Background: While it is well-known that antibodies and antigen-specific T cells play important roles in vaccinemediated protection against typhoid fever, we have yet to understand the protective mechanisms. In a recent vaccine trial we uncovered significant differences in the transcriptional response to two live attenuated oral typhoid vaccines. Gene transcriptional modules linked to NK cells were positively enriched in the response to Ty21a, which provided 35% [95% CI -5 to 60] protective efficacy against experimental challenge, and negative enrichment scores were observed in response to the less protective experimental vaccine M01ZH09 (protective efficacy 13% [95% CI -29 to 41]).

Methods: *In vitro* stimulation of peripheral blood mononuclear cells (PBMCs) with live attenuated *S.* Typhi vaccine strains Ty21a and M01ZH09 followed by flow cytometry for NK cell activation was performed to validate differences in transcriptional profile observed in the vaccine trial. Re-exposure of PBMCs from vaccinated individuals to *S.* Typhi with flow cytometric detection of markers of NK cell activation and functional activity is being performed currently. These experiments investigate whether the capacity of NK cells to respond to re-exposure associates with responses to experimental challenge four weeks after vaccination.

Results: Our data show an increased capacity to activate NK cells in a mixture of PBMCs *in vitro* associated with the more protective vaccine strain Ty21a compared with the less protective vaccine strain M01ZH09. Ongoing experiments may associate the capacity to respond rapidly to re-exposure to *S.* Typhi with clinical outcome parameters such as time to diagnosis.

Conclusions: Our transcriptional data from a recent vaccine study strongly suggests a role of NK cells in response to vaccination against typhoid fever. Validation of these data in *in vitro* experiments indicates an association of NK cell responses with the more protective vaccine, Ty21a, and may associate with clinical parameters measured following experimental challenge.