Investigating Gut Cellular Immunity in a Controlled Human Infection Model of Typhoid Fever

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Background: T lymphocytes are crucial to clear *Salmonella* infections, however it is unclear whether infection leads to the differentiation of gut resident pathogen specific memory T cells capable to confer long-term protection. Our study investigates gut mucosal and peripheral T cell responses in healthy adults recruited in a *Salmonella* controlled human infection model. We hypothesize that T cell responses at the site of infection - the gut mucosa - might provide a more robust cellular correlate of protection than peripheral blood responses only.

Methods: Endoscopic duodenal biopsies and peripheral blood samples were collected from participants at baseline and 4-7 weeks after challenge with a single oral dose of live *S.* Typhi or Paratyphi A. Mononuclear cells were isolated in parallel from intestinal biopsies and blood. Three different *ex-vivo* infection models were undertaken to determine the frequency of *Salmonella* specific T cells. $CD4^+$, $CD8^+$, MAIT and gamma delta T cells were assessed by flow cytometry for their capability to produce cytokines (IFN-γ, TNF-α, IL-17 and IL-2) and up-regulate the activation/memory marker CD40L.

Results: Upon *ex-vivo* infection the predominant cytokines produced by intestinal $CD4^+$ cells are TNF-α and IL-17, conversely blood $CD4^+$ cells produce more IFN-γ. Prior to challenge, the frequency of circulating antigen specific IFN-γ producing $CD4^+$ cells appears to correlate with previous exposure to *Salmonella*. The number of antigen-specific cytokine-producing cells increased post-challenge, with a pronounced increase in the fraction of $CD4^+$ cells upregulating CD40L in response to antigen stimulation. A larger frequency of antigen specific cells was observed in both mucosa and blood from individuals who developed enteric fever upon challenge than those who did not. Mucosa resident gamma delta and MAIT cells were less responsive to *in vitro* bacterial stimulation when compared to peripheral blood cells.

Conclusions: Our *ex-vivo* infection model has enabled us to characterise changes in the frequency of *Salmonella* activated T cells isolated from gut mucosa and peripheral blood before and after human controlled infection with *Salmonella* Typhi and Paratyphi A. Moreover, distinctive patterns of cytokine producing cells have been identified in both compartments. Ongoing analysis may offer insights into possible correlates of protection or associations with clinical presentation.