PhD outline for Jules Thorn PhD Scholarship, Dr Melita Gordon

Background

Invasive *Salmonella* disease is caused both by typhoidal serovars (*Salmonella Typhi* and Paratyphi) to cause Typhoid fever in immunocompetent humans, and by non-typhoidal serovars (*Salmonella Typhimurium*, Enteritidis and others) in immunocompromised individuals, most notably with malnutrition, malaria or HIV in Africa. Vaccination strategies against invasive *Salmonella* disease include whole killed vaccines, live oral attenuated vaccine, conjugate polysaccharide vaccines, or protein vaccines. Existing licensed vaccines for Typhoid fever (Ty21a, polysaccharide Vi vaccine) are suboptimal, and new candidate live oral and conjugates vaccines are under development. There are currently no human vaccines for invasive non-typhoidal Salmonella, although attenuated live oral and conjugate and protein vaccines are in development (Maclennan, Novartis vaccines for Global Health; Finlay laboratory, Vancouver; Levine laboratory, CVD Baltimore). Dr Gordon is a current Gates consortium collaborator with the Novartis and Vancouver vaccine groups.

The most important protective target antigens for protection against invasive typhoid or non-typhoidal Salmonella disease are not known, and the relative importance of humoral or cellular mechanisms of immunity for effective protection are also not understood. Although there are several animal models for non-typhoidal *Salmonella* disease, they do not accurately replicate the setting of the immunocompromised human host. *S. Typhi* is entirely human-restricted, largely limiting investigation of protective correlates to human tissues.

Dr Gordon’s work has previously focused on the host inflammatory and immune response to invasive *Salmonella* disease, the cellular and humoral defects in immunocompromised human hosts that are associated with invasive Salmonella disease, and the investigation of micro-evolved pathovars of Salmonella that may have increased virulence in the immunocompromised host.

More recently, she has been directly investigating the human gut mucosa, sampled at endoscopy in healthy volunteers vaccinated with the Ty21a live oral typhoid vaccine compared to controls. This has defined antigen-specific adaptive responses T-cell subsets and humoral responses in both the gut mucosal and systemic compartments, allowed direct correlation of peripheral markers of response with gut mucosal responses, and validated surrogate peripheral blood markers of gut mucosal cellular responses. It now remains to determine the functional importance of these responses in relation to protection against invasive Salmonella disease in human tissues.

**Hypothesis:** Vaccination with Ty21a and with Vi polysaccharide vaccines will result in very distinct protective immune responses. Dissection of the nature and functionality of these responses will help to understand the most important immunological determinants of protection.

**Aims:** To understand the relative contribution of cellular and humoral responses, and the functionality of the immune response following vaccination for Salmonella Typhi using currently available vaccines.

**Planned work:**

Healthy volunteer adults will be offered vaccination with either the polysaccharide Vi vaccine or the live oral Ty21a vaccine. They will be compared to healthy unvaccinated volunteers and to individuals known to have had naturally-acquired typhoid fever.
The development of the cellular and humoral response in peripheral blood over 6 months will be studied using flow cytometric analysis of antigen-specific T-cell responses, and ELISA for IgG, IgM and IgA antibody production. The functionality of the peripheral immune response will be further studied by:

- Analysis of the development of peripheral T-cell gut homing markers that Dr Gordon laboratory has already shown to be correlated with a gut cellular response, indicating mucosal protection (β7, CCR9)
- Changes in the functionality of the T-cell response, particularly the contributions of IL2, IFNγ and TNFγ to the cellular response.
- Gel electrophoresis and immunoblotting followed by mass spectrometry to assess the targets of the humoral response.
- Assessment of the functionality of the whole and fractionated serum antibody response will be assessed using a serum/antibody bactericidal assay with and without complement, and using opsonisation studies to assess internalisation and intracellular killing of S. Typhi by human macrophages. The impact of priming with IFNγ, or of co-culture with lymphocytes from vaccinated individuals will be assessed. These assays are familiar in Dr Gordon’s laboratory, but will require category 3 facilities, which will be available to the candidate in the University of Liverpool or the CTID of the Liverpool School of Tropical Medicine, according to the appropriate supervisory structure.

Potential collaborations and co-supervisors within University of Liverpool:
- Professor Jay Hinton, Salmonella molecular pathogenesis and regulation, Institute of Integrative Biology, University of Liverpool
- Professor Stephen Gordon, Mucosal immunology laboratory, LSTM
- Professor Aras Kadioglu, Bacterial pathogenesis models, Institute of Infection and Global Health, University of Liverpool

Funding
The studentship is funded with 3 years of UK PhD fees, 3 years of UKRC PhD stipend, plus annual consumable costs., by the Sir Jules Thorn Trust.

The successful candidate
Will show excellence in their academic performance to date, and will preferably hold a Masters degree
Will ideally have experience in cellular tissue culture, flow cytometry and immunological methods including ELISA.