Project 1

Title: To investigate the role of surface layer proteins in the pathogenesis of Clostridium difficile infection

Hypothesis: This project addresses the hypothesis that surface layer proteins (SLPs) are virulence factors for Clostridium difficile infection (CDI) and that the ability of the immune system to recognise these proteins and the resulting immune response, are key factors in both susceptibility to and severity of infection. The project aims to determine whether the outcome of CDI is dependent on recognition of SLPs through TLR4 (an innate immune receptor) and subsequent activation of innate and adaptive immune responses for clearance of the pathogen. This will be achieved by the following 3 specific objectives:

1. To examine the effects of SLPs from different strains of C. difficile on activation of innate and adaptive immunity.
2. To examine C. difficile infection in vivo in the absence of TLR4 recognition of SLPs.
3. To determine the intracellular pathways activated by SLPs.

Methodology: This project comprise primary cell culture of key cell types involved in recognition of pathogens and the generation of appropriate host responses to them. This includes isolation and differentiation of primary murine intestinal macrophages, dendritic cells and specific T helper cell subsets. Each of these techniques is necessary for the determination of a host response to infection. The technology, flow cytometry, is at the core of cellular immunology and is necessary to examine key immune receptors for both recognition of pathogens and for immune cell interactions during an immune response.

Expected output: The data generated in this study will provide information regarding: 1) the potency of the immune response to SLPs isolated from different strains of SLPs, 2) in vivo data on the role of SLP-TLR4 interaction in the pathogenesis of CDI and it will also determine if the strength of the immune response to SLPs determines the severity of CDI, 3) also important information regarding the mechanism by which SLPs activate innate immunity.
Project 2

Title: Role of inflammasomes in cytokine production and host defense against cerebral malaria

**Hypothesis:** Inflammasomes are intracellular multi-protein complexes that are emerging as key regulators of the innate immune response. Deregulated inflammasome activity has been linked to autoimmune diseases. In addition, the critical role of inflammasomes in regulating inflammatory responses and host defense against bacterial, viral and fungal infection is emerging, although the importance of the different inflammasomes and their activation mechanisms during parasitic (protozoan) infections including malaria is less clear.

The underlying hypothesis of this proposal is that hemozoin, genomic DNA of the parasite and other protozoan molecules can induce fever and inflammation in CM by activating inflammasomes in phagocytes. Hence, the overall goal of this project is to characterize the role of distinct inflammasomes in modulating systemic inflammation and fever in mouse models of cerebral malaria. This will be achieved by determining the following two sub-aims: 1) what molecules from the parasite activate inflammasome complexes to cause inflammation and, 2) what are the specific inflammasome complexes that are ligated by these microbial products.

**Methodology:** Characterize the response of mice and immune cells that are deficient for specific inflammasome components to cerebral malaria and stimulation with *Plasmodium* components.

**Expected outcome:** Malaria represents one of the world's most common infectious diseases and affects millions of people worldwide. The research presented in this project will allow us to gain a better understanding of the molecular mechanisms by why *Plasmodium* parasites cause disease, and will extend our knowledge on the roles and activation mechanisms of the distinct inflammasomes during parasitic infections. This may lead to the development of improved therapies and diagnostics that might cure or help prevent malaria infection.
**Project 3**

**Title:** Study of immune and inflammatory responses at invasion of the human colonic tissue by *Cryptosporidium parvum, Blastocystis spp.* and *Giardia lamblia* and the impact of this response on the tissue dissemination of the parasites

**Hypothesis:** Invasion of the human tissue by motile cells is a common process for various biological events; for instance, during the recruitment of the immune cells, the metastasis of tumour cells and the dissemination of parasites. Study of the molecular mechanisms controlling the behaviour of those cells in their natural environment is of key importance for public health. As *in vitro* analyses have been well developed and exploited, but the *in vitro* or findings with culture-adapted materials are not true representative of natural conditions. Thus recently, we have established an ex-vivo model to investigate both parasite behavior and the human tissue response that provides new insights in the molecular mechanisms involved in the early steps of human colon invasion by *E. histolytica*. Consequently, in this project, the interaction between different parasites such as *Cryptosporidium parvum, Blastocystis spp.* and *Giardia lamblia* and intestinal explants from humans (ex-vivo model) will be assessed. We will study invasive mechanism of these parasites on human intestinal barrier, combining a pathophysiological approach and our expertise on these parasites. Utilizing a human colonic organotypic culture, the virulence factors of these parasites and responses of the colonic tissue during the parasite invasion will be analyzed. For dynamic studies of parasite-intestine interaction and invasion, two-photons video microscopy of the invasive process will be performed with human explants.

This will be achieved by the following objectives:

(i) to explore the early steps of tissue invasion, (ii) to determine the patterns of immune response in these explants after incubation with *E. histolytica* and (iii) to analyze the impact of this response on the intestinal dissemination of the parasite.

**Expected results and impacts of the proposed research:** This project involves novel methodologies allowing *in vivo* imaging allied to molecular characterization of parasitic and host components. It should thus make a substantial contribution towards the determination of the rules governing *C. parvum, B. hominis* and *G. lamblia* invasiveness in the tissues. By combining the analysis of parasite invasiveness and host response, we bridge the molecular and physiopathology domains, which have
underpinned rapid advances in the control of other infectious diseases. Our expectations are: the identification of the spatio-temporal rules of infection by recognizing adhesive factors, receptors, toxic compounds and the cells from the immune system activated early on in infection. The identification of major cellular components of the innate immune response would show the involvement in the invasive process of *C. parvum, B. hominis* and *G. lamblia.*