**xMAP Connect Abstract**

**Multiplex immune assays to study immunity to malaria infection and vaccines**

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The identification of immune correlates of protection and risk against complex infectious diseases such as malaria is particularly challenging because the parasite’s proteome has over 5,000 potential proteins, some of them polymorphic and/or variant. Naturally-acquired immunity to *Plasmodium* parasites is mainly mediated by IgG antibodies but the subclasses, epitope targets and effector functions have not been unequivocally defined. In addition, the role of cellular immune mediators has been less characterised. Multiplex high-throughput miniaturized platforms can be used to dissect the type and specificity of immune responses mediating immunity to malaria towards developing more effective vaccines to control the disease. In addition, they can be applied to elucidate the mode of action of experimental vaccines.

We have developed a series of protocols based on the xMAP technology to measure antibodies of multiple isotypes and subclasses and against several infectious diseases and vaccine antigens to understand naturally-acquired and experimentally-induced immunity to malaria. In addition, we have optimised commercially available kits for the quantification of cytokines, chemokines and growth factors present in plasma or serum samples, or in culture supernatants after in vitro stimulation of immune cells with parasite antigens. This has been applied in large immune-epidemiological studies to assess the immunogenicity and mechanism of action of experimental malaria vaccines and adaptive immunity in the field, taking into account the responses to other infections and, in young infants, prenatal exposures in utero and maternally-derived antibodies.

We have shown that antibody responses to malaria include all isotypes with predominance of cytophilic IgG1 and IgG3 subclasses. IgG2 responses were associated with risk of clinical malaria while IgG1 and IgG3 were associated with protection. Regarding T cell responses, a predominance of TH2 over TH1 cytokines in response to vaccination appears to be associated with malaria risk. Such biomarkers shed light into the mechanisms that may be acting in protection or risk against malaria. In addition, complex interactions between immune responses to vaccines and to natural parasite exposure were identified that may impact the development of protective immunity in vaccinated subjects living in endemic areas.

The complexity of malaria and other diseases require that the breadth of the antigenic specificities as well as the diversity of immune effectors are fully characterised in a multidimensional manner and that complex analytical tools including multivariable analysis and machine learning algorithms are applied to dissect the immune responses that are most relevant in immunity. This information is essential for a rational development of second-generation more effective vaccines.

The xMAP technology protocols developed for malaria are readily applicable to the study of other infections that are prevalent in low income countries and that affect the health of the vulnerable populations, as well as other biomarker studies of diseases of global impact.