Discussion:

- *Plasmodium falciparum* IgG and IgM were detected in plasma samples of immune donors. This confirmed exposure to parasite of the immune donors and increased the chances of obtaining circulating anti-malaria B– cells
- Method 2 which involved isolation of total Malaria antigens specific B cells by using Biotin-Dynabead Streptavidin was successful as it gave higher IgG levels compared to method 1 (isolation of iRBC surface reactive B cells).
- Cytokine combinations IL2+IL10+IL15 seemed to be the best combination as it gave the highest titre of IgG (cytokine combination 19 as seen in figure c)

Conclusion:

The produced Malaria specific antibodies from this study can be of assistance in understanding how the immunologic memory against *P.falciparum* is functioning as well as screening for parasite antigens and identifying potential vaccine candidates.

Acknowledgement:
I would like to thank my supervisors Kristina Persson and Sreenivasulu Reddy for their great support throughtout my project.
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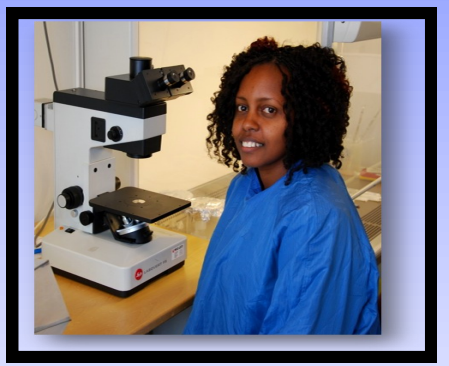
Karolinska Institutet

Linköpings Universitet

EBV immortalization and characterization of Malaria specific B cells from immune donors

Florence Urio
Masters thesis 2012



Supervisors:
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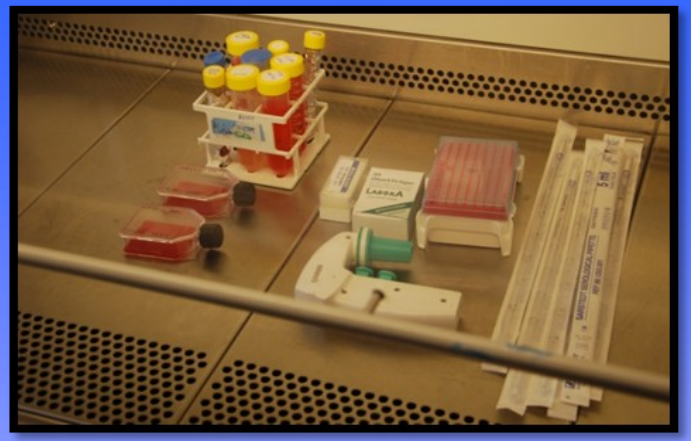
Background:

-Malaria resulting from *Plasmodium falciparum* infection is the major cause of mortality and morbidity. The World Health Organization (WHO) reports that annually malaria causes over 300 million incidences of acute illness and more than one million deaths. Majority of these deaths occur in the poor countries of the tropics of which children and pregnant women have the highest mortality rate.

-Many on-going researches are aiming at finding a vaccine against malaria, mainly due to the fact that resistance to anti-malarial drugs is increasing rapidly. In this study, Epstein Barr Virus was used as a tool to immortalize B cells. The virus does not damage the B cells but instead it infects and activates the B lymphocytes *in vitro*.

Aim of the project:

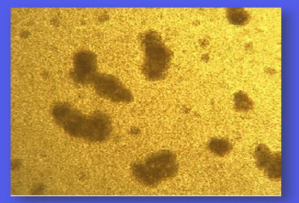
The aim of the project was to establish and characterize *Plasmodium falciparum* specific B cell lines (Lymphoblastoid cell lines) using an EBV immortalization protocol.



Methods:

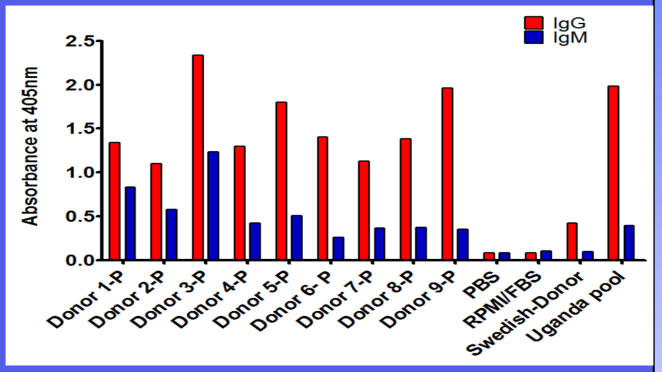
- Ficoll-hypaque gradient centrifugation was used to isolate lymphocytes from 9 immune donor
- B cells were immortalized by EBV
- Magnetic activated cell sorting method was used to obtain the Schizont extract from FCR3S1.2 strain
- Plasma samples from immune and non immune donors were tested for *Plasmodium falciparum* specific antibodies
- **Method 1:**Schizont extract from FCR3S1.2 strain was incubated with immortalized B cells then purified the Malaria specific B cells by MACS method.
- **Method 2:** Labeled parasite antigen-DSB-X biotin with immortalized B cells were incubated then Dynabeads Streptavidin were used for isolation of Malaria specific B cells
- Parent cell lines and Malaria specific B cell lines were stimulated with combinations of IL2, IL6, IL10, IL15 and IL21
- -ELISA test- detected *Plasmodium falciparum* specific antibodies

Results:



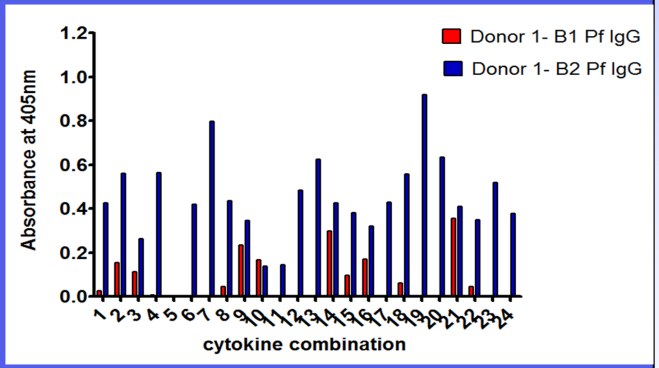
A

- *Aggregation of lymphoblastoid cell lines which indicates successful transformed B cells*



B

- *Detection of P.falciparum specific IgG and IgM in donors plasma samples*



C

- *Detection of Malaria specific IgG in donor 1 from method 1 & 2 after cytokine stimulation*
- *B1– Method 1 and B2 - Method 2*