Developing a Human Organoid System for Malaria Research

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Background

Malaria, caused by the Plasmodium parasite is transmitted via the bite of an infected female Anopheles mosquito. It carries a massive disease burden, killing 627 000 people in 2020[1]. Sterile immunity cannot be developed, however disease tolerance to severe malaria can be developed[2] . However, children in malaria endemic areas appear more susceptible to other diseases and show reduced responses to vaccines[3] . Effective immunity to malaria requires the production of high-affinity antibodies, which are made by plasma cells in large complex cellular structures called Germinal centres (GC).

Germinal centres (GC) are found in secondary lymphoid organs like the bone marrow. They are critical as the plasma and memory B cells found within them produce high affinity antibodies[4] **(Figure 1)**. Disruption to the formation of GCs during malaria infection may explain the evidence of immune suppression in malaria endemic areas and why anti-parasite immunity is slow to develop, but there is currently no model to test this.

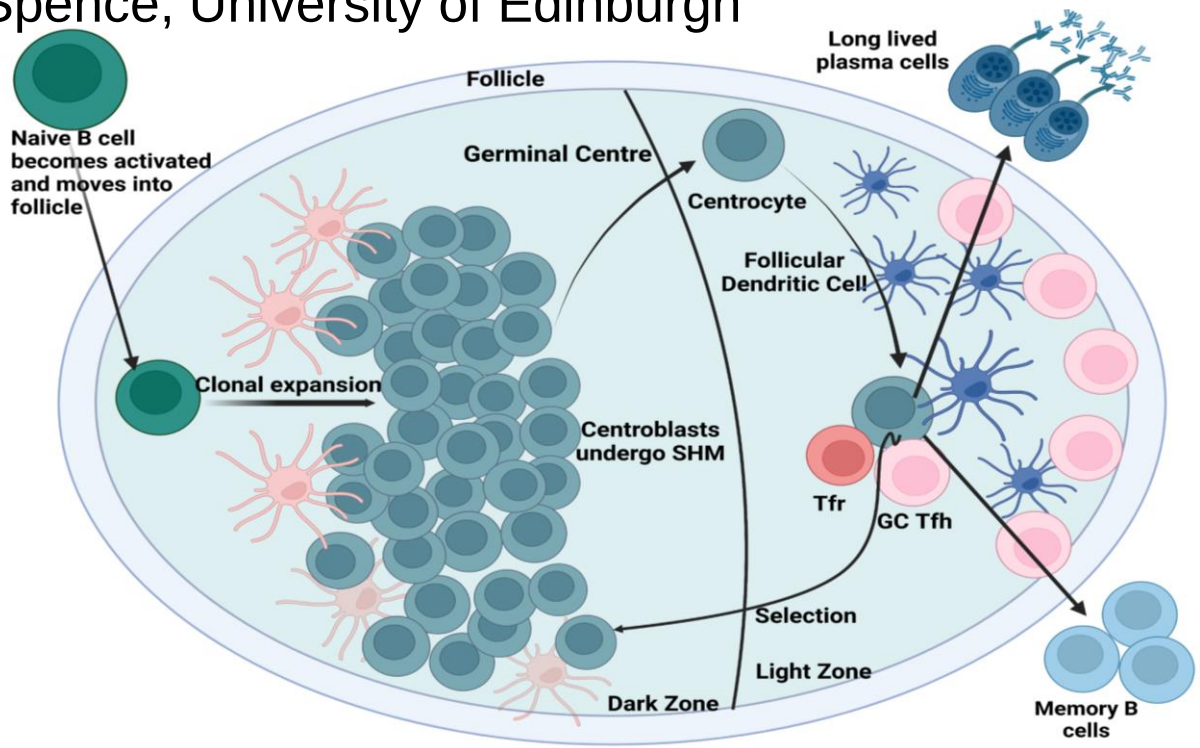

Figure 1: Generation of high affinity antibodies in germinal centre
Centroblasts undergo clonal expansions and somatic hypermutation in the dark zone before moving to the light zone for selection. Selection is aided by follicular dendritic cells presenting antigen. Upon selection and signals from T follicular helper cells (Tfh), the centroblasts endure further expansion and somatic hypermutation before exiting the GC as plasma cells or memory B cells that produce high affinity antibodies [4]. (Figure adapted from [4]).

Objective: To create a model system to examine GCs before and after malaria infection to understand whether immune suppression occurs.

Approach

- Bone marrow cells from CMV+ donors were cultured *in vitro*
- Either unstimulated (US) or CPI (protein with antigen from CMV, influenza and parainfluenza virus) stimulated.
- After 12 days, supernatant was taken off to analyse antibody production by ELISA
- Single cell suspension of organoids were prepared
- These were stained with antibodies for flow cytometry analysis.

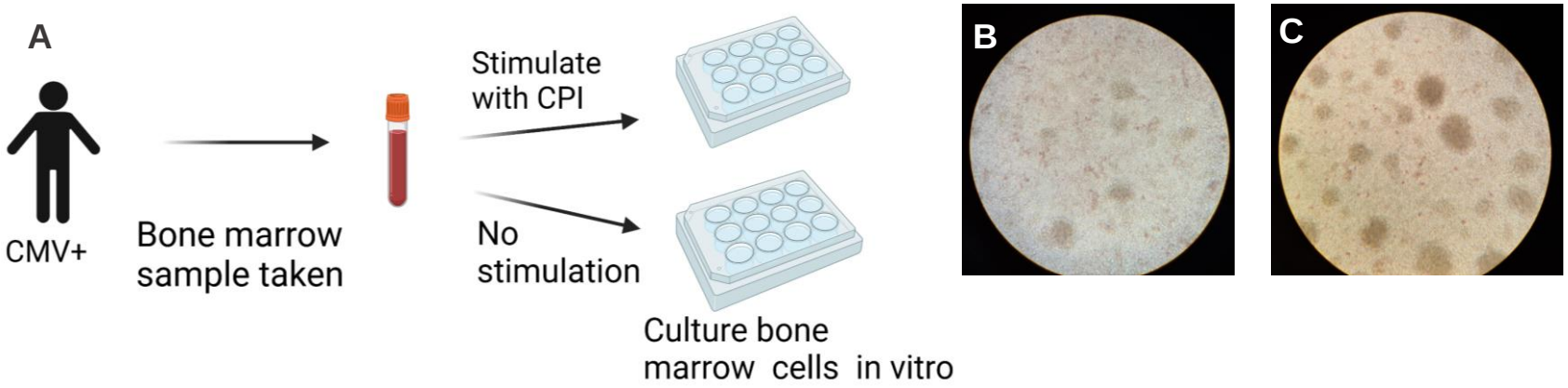

Figure 2: Experimental Approach
A:Schematic of experimental approach **B:** CMV+ unstimulated organoids day 11
C: CMV+ CPI stimulated organoids day 11

Results

- **Germinal centre formation was present within the organoids**
- To conclude germinal centres had formed, needed to see an increase in plasmablasts and GC B cells in stimulated compared to unstimulated sample as these cells are indicative of GC formation
- Flow cytometry (**Fig. 3**) showed clear increases in plasmablast and GC B cell populations
- Proportionally, the frequency of plasmablasts increased starkly in the stimulated samples compared to the unstimulated (**Fig. 4A**)
- ELISA analysis showed antibody production, with a higher amount being made in the stimulated samples (**Fig. 4B**)

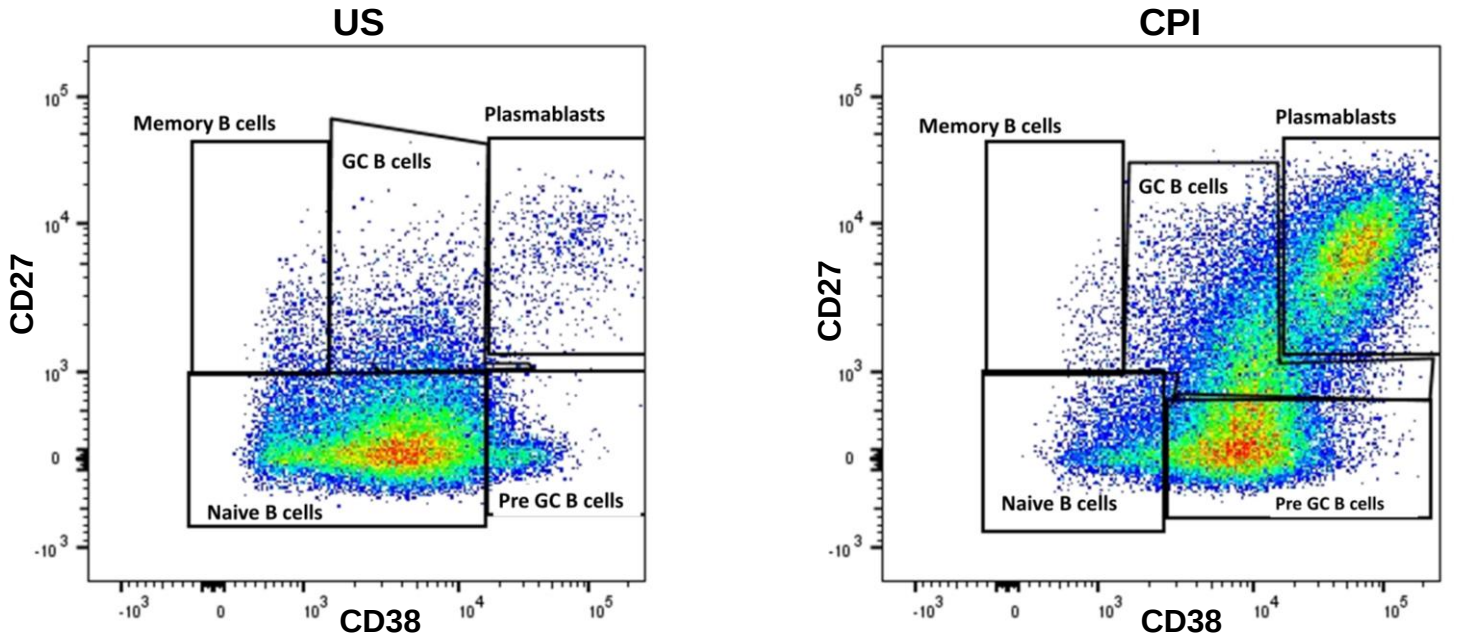

Figure 3: Flow cytometry results
Gated on singlets, live and CD19+ cells to show different B cell populations in organoids.
Cells were defined as: Naïve B cells CD27- CD38-, Pre GC B cells CD27+ CD38-,
GC B cells CD27+ CD38+, Plasmablasts CD27+ CD38++, Memory B cells CD27- CD38+

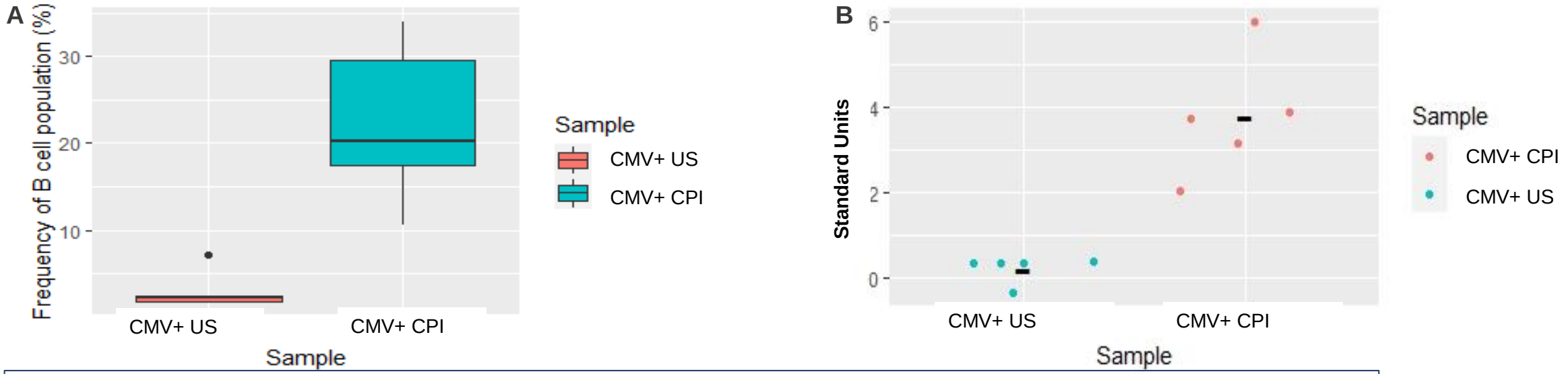

Figure 4: Frequency of plasmablasts of B cell population (A), ELISA analysis results (B)
A: Frequency of plasmablasts present in CD19+ B cell population from 5 samples
B: Results of ELISA analysis to check for antibody production in standard units. Black line represents mean for each group

Future Research and Importance

- Now have a model to test whether malaria disrupts GCs and if this can explain immune suppression (**Fig. 5**)
- Using controlled human malaria infection (CHMI) trials can sample bone marrow prior to infection and after infection
- Culture into organoids
- Is there a difference before/after malaria?

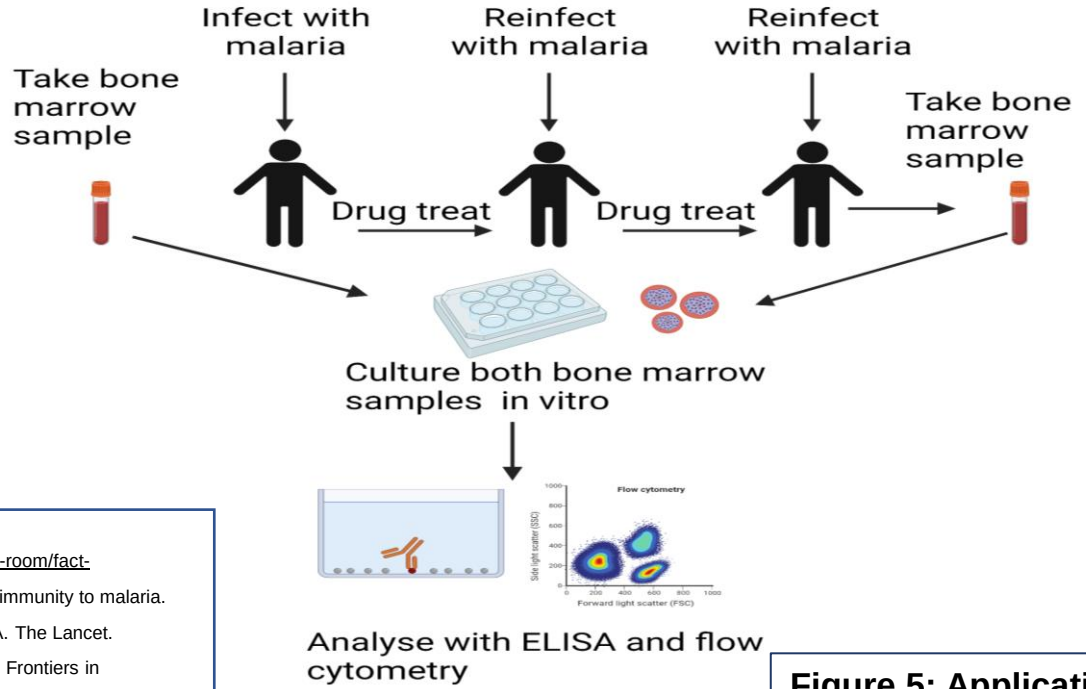

Figure 5: Application in future research

References
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3.Williamson W, Greenwood B. IMPAIRMENT OF THE IMMUNE RESPONSE TO VACCINATION AFTER ACUTE MALARIA. The Lancet. 1978;311(8078):1328-1329.
4.Stebegg M, Kumar S, Silva-Cayetano A, Fonseca V, Linterman M, Graca L. Regulation of the Germinal Center Response. Frontiers in Immunology. 2018;9.