Cerebral malaria and CR1 location

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What is the problem?

Cerebral malaria (CM), a severe manifestation of malarial infection which affects the brain, is a life-threatening disease that represents a major health problem particularly in Sub-Saharan Africa, Southeast Asia, and South America. With over half a million cases annually, children in sub-Saharan Africa are the most affected case of CM and it is a major cause of death among those children (see Figure 1). The disease develops when the parasite that causes malaria, *Plasmodium falciparum*, sticks to the lining of the brain's blood vessels, leading to coma and death. Surviving patients have an increased risk of neurological and cognitive deficits, behavioural difficulties and epilepsy, making cerebral malaria a leading cause of childhood neuro-disability in these regions.

Figure 1 The devastating impact of cerebral malaria on children. Credit for image: Dr Olivia Swann.

What are we interested in?

Different versions of a protein, called CR1, have being associated with increased survival against CM. We had previously found that the CR1 protein is present in and on the surface of human brain endothelial cells (HBECs), which are the cells that line the inner surface of blood vessels, but we didn't know exactly where within the cell CR1 was located. The aim of this project was to determine the specific location within the HEBCs of the CR1 protein. This knowledge might shed light on its role in cellular processes which could lead to the development of therapies to protect children against cerebral malaria in the future.

What did we do?

We used "glow-in-the-dark" markers bound to a set of antibodies, each of which recognised and bound specifically to different compartments (organelles) of the cell or to the CR1 protein. The organelles we looked at were the endosomes, lysosomes, endoplasmic reticulum, Golgi apparatus and mitochondria. The markers were of different colours which let us investigate whether the CR1 protein co-localises with any of these intracellular organelles. We used a green marker for the CR1 protein and a red marker for the organelles. This let us see where CR1 was being stored in the cell and the location of the organelles. Where the two different dyes overlapped, the cells shone yellow and this represented the organelle to which CR1 co-localises. We also stained the nuclei of the cells (the organelle where most of the cell's DNA is stored) in blue.

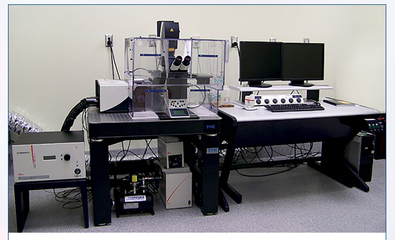

Figure 2 A confocal microscope.

We did this for both immortalised HBECs (which grow indefinitely in the lab) and for primary HBECs which had been isolated directly from human tissue and the experiments were imaged using a confocal microscope (see Figure 2).

What did we find?

When we visualised the HEBCs under the confocal microscope we saw staining for the CR1 protein consistent with it being located within the cells and at the cell surface (see Figure 3 – 7). Each organelle gave its own specific staining pattern as expected (see Figures 3-7). Staining for the CR1 protein did not colocalise with the mitochondria (see Figure 3), endosomes (see Figure 4), lysosomes (see Figure 5) or endoplasmic reticulum (see Figure 6). However, it consistently colocalised closely with the Golgi apparatus (see Figure 7).

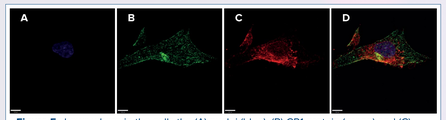

Figure 5 shows where in the cells the (A) nuclei (blue), (B) CR1 protein (green) and (C) lysosomes (red) are located. (D) Overlaying B and C indicate CR1 and the lysosomes do not co-localise.

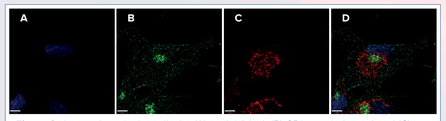

Figure 3 shows where in the cells the (A) nuclei (blue), (B) CR1 protein (green) and (C) mitochondria (red) are located. (D) Overlaying B and C indicate CR1 and the mitochondria do not co-localise.

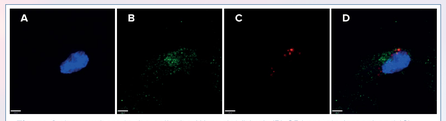

Figure 6 shows where in the cells the (A) nuclei (blue), (B) CR1 protein (green) and (C) the endoplasmic reticulum (red) is located. (D) Overlaying B and C indicate CR1 and the endoplasmic reticulum do not co-localise.

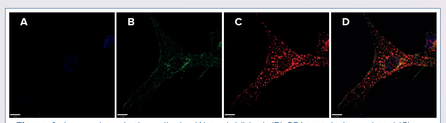

Figure 4 shows where in the cells the (A) nuclei (blue), (B) CR1 protein (green) and (C) endosomes (red) are located. (D) Overlaying B and C indicate CR1 and the endosomes do not co-localise.

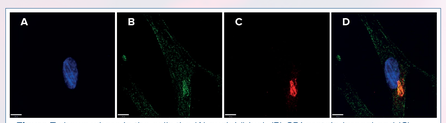

Figure 7 shows where in the cells the (A) nuclei (blue), (B) CR1 protein (green) and (C) Golgi apparatus (red) are located. (D) Overlaying B and C indicate CR1 and the Golgi apparatus co-localise (yellow).

What does it mean?

Our experiments indicate that the CR1 protein co-localises with the Golgi apparatus in HBECs. The malarial parasite infects red blood cells and it is known that these infected red blood cells bind to other non-infected cells in the body, exacerbating disease. Infected red blood cells have been shown to bind strongly to CR1. If the CR1 we have seen in our experiments in the Golgi apparatus of HEBCs, is transported from inside the cells to the cell membrane it might act as a binding partner for malaria-infected red blood cells, with different versions of the CR1 molecule resulting in different strengths of binding, accounting for observed variations in disease severity. This raises the possibility that blocking or reversing CR1 binding by infected cells to HEBCs could be one avenue for therapy.

CR1 is known to function as part of the immune system (which protects us from infections and harmful agents). It does this, in part, by being part of a complex cascade of protein reactions, called the complement system. One effect of the complement system is to punch holes in the surface of cells, including infectious agents. Another role of the complement system is to form complexes with harmful agents which can then be destroyed by cells of the immune system.

It might be that CR1 from HBECs is secreted from the HBECs into the microcirculation of the human brain where it could form complexes with the harmful *Plasmodium falciparum*, leading to destruction of the parasite. Finally, a third possibility is that it might be acting to protect the HEBCs from attack by the complement system. If so, soluble CR1 could be a potential therapy for cerebral malaria.

Future work is required to examine in more detail the possible role of CR1 in the brain during CM, and the potential for CR1-related therapeutics.

Who am I?

I am in my final year at the University of Edinburgh working towards a BSc in Biochemistry. I did this research project in the summer of 2016 and I would like to thank Medical Research Scotland for the funding support and my supervisors, Professor Alex Rowe and PhD student Olivia Swann, for their help and encouragement throughout the project. Although I am still considering my career choices, following my degree, I would like to pursue a PhD in biochemistry or a related area.

Alexandros was supervised during his Vacation Scholarship project, *"Where is complement receptor 1 stored in human brain endothelial cells?"*, by Professor Alex Jane Rowe and Dr Olivia Swann.