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Title: Complement receptor 1 is the host erythrocyte receptor for Plasmodium falciparum PfRh4 invasion ligand.
Authors: Wai-Hong Thama, Danny W. Wilson, Sash Lopaticki, Christoph Q. Schmidt, Patience B. Tetteh-Quarcoo, Paul N. Barlow, Dave Richard, Jason E. Corbin, James G. Beeson and Alan F. Cowmana
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Rationale for Choice

Malaria is one of the most infectious diseases killing 2-3 million people every year. It is transmitted by the bite of *Anopheles* mosquito infected with *Plasmodium*. Currently there are drugs available for treating malaria. However drug resistance is now developing against all classes of antimalarial drugs. Additionally, there is no current vaccine against malaria. Therefore more vaccine and drug designs are required in preventing this disease.

There are five species of *Plasmodium* however most deaths are caused by *falciparum*. *Plasmodium falciparum* is an obligate parasite therefore its ability to invade the host erythrocytes is crucial. The merozyte forms of *P.falciparum* invade the erythrocytes using a multistep process. The steps are dependent on interactions between the parasite invasion ligands and their respective human erythrocyte receptors. In this study, the researchers discovered the host erythrocyte receptor for the ligand PfRh4. This ligand is essential for the sialic acid-independent pathway [1]. Understanding those interactions can lead to designing drugs and/or vaccines. For this reason, this paper was chosen.

In Australia there are programs which screen and treat malaria in migrants and refugees from tropical areas. “However this does not apply to returning Australian nationals, who represent a much larger potential source”. [2] Approximately 250,000 Australians visit countries with malaria annually. Therefore malaria is mainly brought into Australia via travellers. In addition, there are detection problems in Australia. These are due to lack of suspicion, lack of undergraduate and postgraduate parasitology teaching and long incubation period which complicate the link between possible transmission and presentation [2]. Therefore Australia can also benefit from the future vaccine and drug designs against malaria.

Comment [U1]: The above three paragraphs are written according to the rule of threes. The three main ideas under the “rationale for choice” are – the epidemiology of malaria, the parasite and how it affects Australia.

For each idea, dot point outline was applied which then became the sentences in the paragraph.

Description of the Findings of Study

The most significant finding of the study is the discovery of a receptor for the parasite ligand PfRh4. To test this, erythrocyte cells were incubated with anti-CR1 antibodies and erythrocyte-binding assay was performed. The researchers then observed the PfRh4 erythrocyte binding was reduced whereas the EBA-175 binding (control) was not affected. EBA-175 is a ligand essential for erythrocyte invasion and it is known to bind to its ligand, glyphorin A. It was also observed that, increasing the amount of anti-CR1 antibodies enhanced the inhibition of PfRh4 binding. More than 90% binding was blocked at an antibody dose of 0.0107 mg/mL.

The researchers also tested if soluble recombinant CR1 can inhibit the binding of PfRh4 to the surface of human erythrocytes. The soluble recombinant CR1 contained the extracellular domain and not the trans-membrane and cytoplasmic domain. Culture supernatant was preincubated with soluble CR1 and was added to the erythrocyte-binding assay. It was observed that, increasing the amount of soluble CR1 blocked the binding of PfRh4 to the erythrocyte surface. More than 90% inhibition was obtained at 0.04 mg/mL. “Probing of the same binding eluates with EBA-175 antibodies resulted” in no effect on EBA-175 erythrocyte binding which showed that, the

inhibition is specific to PfRh4. This showed that, CR1 serves as a receptor for the parasite's erythrocyte invasion ligand, PfRh4.

The study also showed that, the levels of PfRh4 binding correlates with CR1 level on the erythrocyte surface. CR1 levels vary between individuals and range from 50 to 1,200 molecules per cell. In Caucasians, a single polymorphism at exon 22 is related with high expression of CR1. Homozygous (HH – high allele) individuals have higher CR1 on erythrocyte surface whereas homozygous (LL – low allele) have less than 200 molecules per erythrocyte and heterozygous (HL) individuals have intermediate CR1 levels. The researchers took 100 individuals blood sample for analysis and found a strong correlation between the percentage of erythrocytes bound to recombinant PfRh4 and the CR1 level on erythrocyte surface ($r2 = 0.83236$).

The researchers looked at the importance of CR1 as a receptor for the PfRh4 invasion by evaluating the invasion of parasites, W2mefΔ175 and 3D7 into neuraminidase-treated erythrocytes. These strains are known to invade neuraminidase-treated erythrocytes. The erythrocytes were treated with neuraminidase in order to stop the sialic-dependent invasion of the parasite. This results in the blockage of invasion by other ligands such as EBA-175. From the experiment, it was observed that, the invasion of both W2mefΔ175 and 3D7 into neuraminidase-treated low-CR1 erythrocytes was reduced compared to neuraminidase-treated high-CR1 erythrocytes. This showed that, neuraminidase treated erythrocyte invasion mainly relies on the PfRh4-CR1 interaction.

They also treated parasites lacking the PfRh4 expression with soluble CR1. This resulted in no reduction in erythrocyte invasion. This is due to the lack of interaction between soluble CR1 and PfRh4.

The study not only shows the receptor for the ligand PfRh4 but also provides a molecular basis for inhibition of invasion.

Comment [U2]: Under the sub topic "description of findings", the main findings of the paper was discussed.

Each paragraph explains one finding only. It does not overlap with the other findings. Hence one idea per paragraph was applied.

Significance of the Findings

The study provides an insight for vaccine design that can use the erythrocyte invasion of the parasite, for the host's benefit. A vaccine that mimics the ligand and has no virulence on the inside can be designed. This will trigger the immune response causing production of antibodies against the parasite but the host will not be infected as there are no virulence factors inside the parasite. Hence when the host encounters the parasite, the immune system will recognise it much more quickly and respond efficiently due to its previous encounter with the parasite. Therefore people travelling to malarious places including Australians can be immunised and transmission can be prevented.

As mentioned earlier, the current malaria drugs are becoming more resistant. Hence it is also important to design new drugs which can effectively cure the disease. Finding the receptor for the ligand PfRh4 also opens doors for drug designs. Understanding ligand-receptor interaction helps in designing drugs that can prevent the interaction. Molecules which physically block the binding of the ligand to the receptor can be designed. This will stop the invasion of the erythrocyte which will prevent the disease. Some Australian travellers do not notify when leaving to malarious places [2]. Hence they have a good chance of being infected. If this occurs, such drugs can be used in treating them and prevent the transmission.

The study also showed that a soluble CR1 can bind to the receptor and inhibits erythrocyte invasion. With more research and analysis, this soluble CR1 can be administered right after a mosquito bite. Ideally, this means the ligand will not be able to bind to the receptor as it will be already occupied by the soluble CR1. Hence the parasite will not survive in the host.

Comment [U3]: The subtopic "significance of the findings" was also structured according the rule of three's.

It has three points; vaccine design, drug design and future research directions.

Under each idea, dot point outline was carried out which became the sentences.

Reference List

1. **Tham WH, Wilson DW, Lopaticki S, Schmidt CQ, Tetteh-Quarcoo PB, Barlow PN, Richard D, Corbin JE, Beeson JG, Cowman AF.** 2010. Complement receptor 1 is the host erythrocyte receptor for Plasmodium falciparum PfRh4 invasion ligand. Proceedings of the National Academy of Sciences of the United States of America **107:**17327-17332.

2. **Andrew Thompson RC, Owen IL, Puana I, Banks D, Davis TM, Reid SA.** 2003. Parasites and biosecurity--the example of Australia. Trends in parasitology **19:**410-416.