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Plasmodium Vivax Binds Host CD98 (SLC3A2) To Enter Immature Red Blood Cells[1]

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INTRODUCTION

- **Malaria** is a leading cause of mortality and morbidity worldwide and causes huge burden on economic and societal development[2]
- Malaria cases increased from 227 to 241 million cases from 2019 to 2020[3]
- Malaria is transmitted by the female Anopheles mosquito
- Curently, five parasite species of Malaria can cause zoonotic disease
- ***Plasmodium falciparum*** and ***Plasmodium vivax*** are the leading causes of malaria, with *P. vivax* being the most common species in Southeast Asia
- Knowledge on erythrocyte invasion is centred around *P. falciparum* due to its ability to invade all subtypes of erythrocytes and thus erythrocytes are easily accessible
- *P. vivax* is not as well studied due to challenges in **in-vitro cultivation** of parasites in red blood cells

AIMS

- Current literature shows that ***P. vivax* merozoites target preferentially target reticulocytes expressing CD71 which is trypsin sensitive**
- However, other studies show that merozoite invasion is **trypsin-resistant**
- Suggests the possibility of an alternative receptor for *P. vivax* merozoites
- To identify a possible alternative receptor other than CD71, on reticulocytes for invasion by *P. vivax* merozoites

RESULTS

1. Identification of CD98, a Reticulocyte Binding Protein

- A differential proteomics screen of CD71+ (susceptible) vs CD71- (non-susceptible) erythrocyte ghost membranes was conducted
  - CD71+ cells were used as it has already been identified in literature
  - CD71- cells were used as a negative control
- **Two criteria were used to select candidate reticulocyte-specific receptors:**
  - Abundant expression on CD71+ immature reticulocytes but not CD71- normocytes
  - Resistance to trypsin treatment
- Proteins highly expressed on CD71+ reticulocytes were identified *(Fig 1b)*
- Some of these proteins demonstrated to be trypsin resistant on flow cytometry
- CD71 (~6 fold) and CD98 (~4 fold) showed significant fold-change reduction in expression levels between CD71+ and CD71- erythrocytes
- **Immunophenotyping, immunofluorescence and western blotting analyses** confirmed the decrease in CD98 expression during erythrocyte maturation

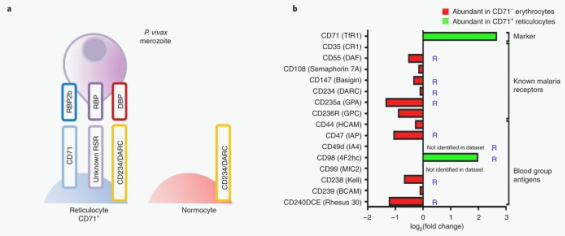

Fig 1a (Left): Schematic showing presence of unknown P. vivax receptor involved in reticulocyte tropism

Fig 1b (Right): Identifying receptors expressed on CD71+ and not CD71- erythrocytes by mass spectrometry

2A. Confirmation of CD98 as a Malaria Target Receptor

- Different *P.vivax* isolates were used in **invasion inhibition assays** *(Fig 2)*
- Anti-CD98 antibodies as well as antibodies to three other erythrocyte membrane proteins -CD240DCE, CD99 and CD147 were added
- Anti-CD98 antibodies significantly annulled the entry of *P.vivax* merozoites into CD71+ reticulocytes with high levels of inhibition (~70%) while the other antibodies had little to no effect on invasion efficiency.

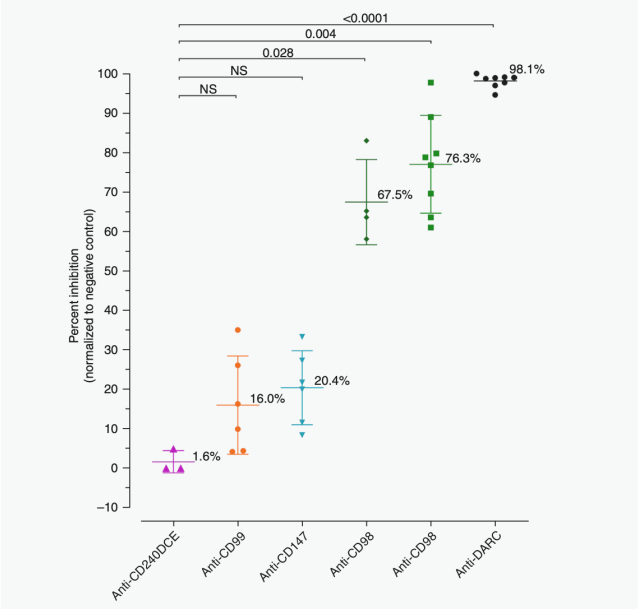

Figure 2: P. vivax inhibiting assay using monoclonal and polyclonal antibodies with anti-DARC antibody as inhibition positive control

2B. Identification of PvRBP2a and CD98 as Binding Partners

- PvRBP2a and CD98 were both **co-immunoprecipitated** *(Fig 3)*
- This shows that **PvRBP2a and CD98 are direct binding partners**
- Direct binding interactions were further confirmed through **ELISA-based binding assays and bilayer interferometry**

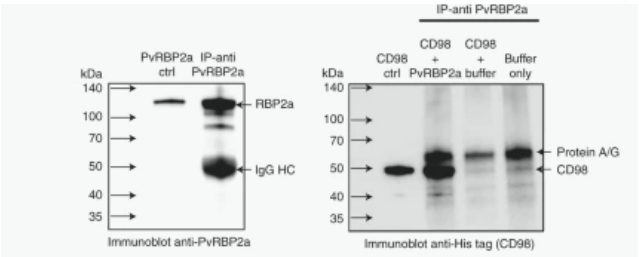

Figure 3: Successful immunoprecipitation of PvRBP2a (lane 2 on left) and CD98 (lane 2 on right)

CONCLUSION

- CD98 was identified as an additional reticulocyte-specific host receptor that is directly associated with *P.vivax* reticulocyte tropism
- **PvRBP2a was identified as the ligand on *P.vivax* which bound to CD98**

DISCUSSION AND APPLICATIONS

- Identification of reticulocyte-specific receptor-ligand pairs can aid **development of continuous in vitro cultures** of *P.vivax* for research
- Given that PvRBP2a can induce strong immunoglobulin G response, it would be useful in **future vaccine research, with the aim of eventually eradicating Malaria**

REFERENCES

1. Malleret B, El Sahili A, Tay MZ, Carissimo G, Ong ASM, Novera W, et al. Plasmodium vivax binds host CD98hc (SLC3A2) to enter immature red bloodcells. Nat Microbiol. 2021 Aug;6(8):991–9.
2. Guinovart C, Navia M, Tanner M, Alonso P. Malaria: Burden of Disease. CMM. 2006 Mar 1;6(2):137–40.
3. World malaria report 2021. Geneva: World Health Organization; 2021.

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