

## Antibodies to PfSEA-1 block parasite egress from RBCs and protect against malaria infection

Recently, an exciting article was published in Science, where Dipak Raj and colleagues have applied several innovative approaches in the search for a rationalized vaccine target <sup>1)</sup>. Crucially they point out that “of the ~100 vaccine candidates currently under investigation, more than 60% are based on only four parasite antigens”, which from a genome encoding more than 5000 proteins, leaves an enormous number of parasite antigens that remain unexplored.

Unlike conventional studies that have sought to define the potential of a known protein as a target for protective immune responses, here the authors took a different approach by screening the plasma of young children who were resistant or susceptible to clinical *P. falciparum* malaria. They identified three proteins that were unique to the resistant children: PF3D7\_1021800, PF3D7\_1134300 and merozoite surface protein-7 (MSP-7).

- Further investigation of the first of these proteins revealed that it is expressed at the late schizont stage within the parasitophorous vacuole, Maurer's clefts and at the infected RBC surface. Not only could specific antibodies reduce parasite growth *in vitro* by up to 74%, but the schizonts remained intact - suggesting that the antibodies were inhibiting schizont rupture. Hence PF3D7\_1021800 has been named *Plasmodium falciparum* schizont egress antigen-1 (PfSEA-1).

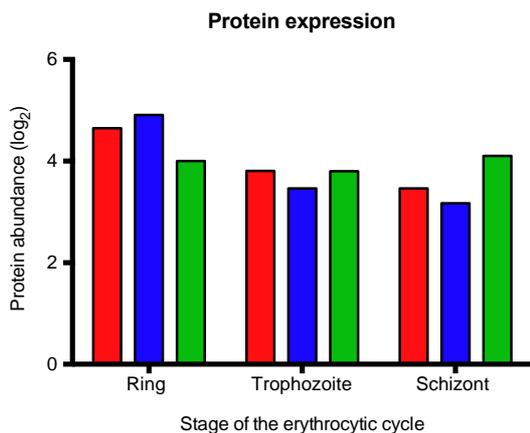
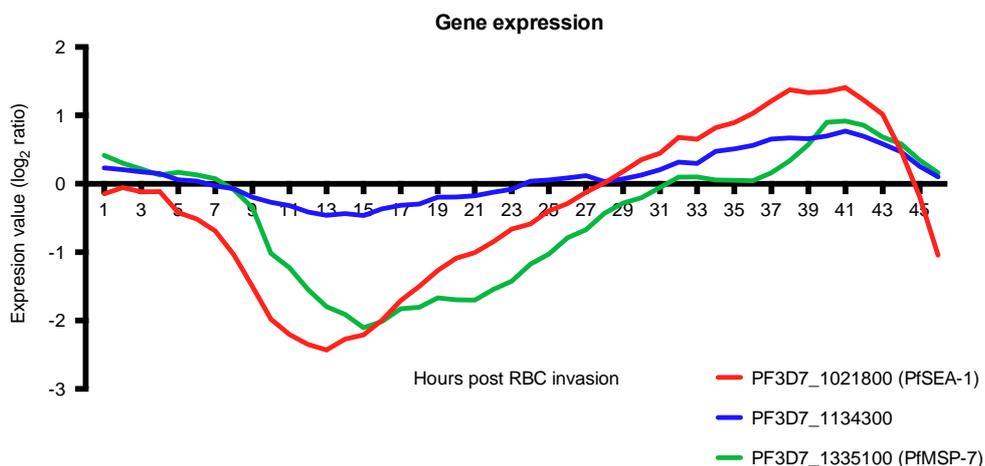
It would be interesting to see how PfSEA-1 interacts with other known mediators of schizont egress such as the protease Sub-1. MSP-7 and the SERA family of proteases are among the known substrates of Sub-1, thought to initiate a cascade within the schizont leading to its rupture and release of merozoites <sup>2)</sup>.

- PfSEA-1 has orthologues across all *Plasmodium* spp and appears to be essential for blood-stage parasite growth since attempts to knock it out were unsuccessful. A conditional knock down approach however was able to reduce PfSEA-1 expression by 75%, resulting in a similar-scale reduction of parasite growth *in vitro* compared to wild-type parasites.
- *In vivo* testing of this protein gave encouraging results as mice directly immunized with *P. berghei* SEA-1, and also those given immune serum by passive transfer, survived for approximately 12 days longer than control mice following lethal *P. berghei* ANKA (PbA) infection. Surprisingly however, the immunised mice were able to reach moderate parasitaemias after a delay of 2-3 days compared to controls and did not appear to resolve the infection. One explanation may be that the schizonts in immunized mice, blocked from egress by anti-PfSEA-1 antibodies, were mechanically ruptured via passage through the spleen so that some merozoites were able to continue to invade red blood cells (RBCs).
- The final encouraging indicator of PfSEA-1 as a potential vaccine candidate is that antibodies to this protein could be detected in the sera of two longitudinally followed cohorts and the levels of these antibodies correlated with protection. Young adult Kenyans with antibodies to PfSEA-1 who went on to develop malaria during the follow up period had approximately half the parasite densities of those without detectable antibodies. Additionally, in a young Tanzanian cohort, all children with detectable anti-PfSEA-1 antibodies had a significantly reduced risk of developing severe malaria complications. Importantly, there was no reduction of risk to severe malaria development in these children when antibody titres to other malaria vaccine candidates were measured, including MSP-1<sub>19</sub>, MSP-3, LSA-N, LSA-C and RAMA-E. This suggests that acquiring antibodies to these well-characterized vaccine candidates is less associated with protection than antibodies to PfSEA-1, at least in the cohorts studied.

As for the other proteins identified in this paper, MSP-7 is known to form a complex with MSP-1 and 6, forming the most abundant component of the merozoite surface coat. Whilst knockout of this protein alone has only shown a partial reduction of invasion *in vitro* <sup>3)</sup> and *in vivo* <sup>4), 5)</sup>, in combination with Plasmepsin-4 disruption, mice were protected from PbA induced ECM, were able to resolve infection and were protected against heterologous challenge <sup>5)</sup>. This suggests that a vaccine containing several of these candidates could have much greater efficacy than the individual proteins alone.

It will be exciting to see what is uncovered about the role of the remaining hypothetical protein PF3D7\_1134300. A quick comparison of expression data available on PlasmoDB suggests that the patterns of transcription for all three candidates identified by Raj and colleagues <sup>1)</sup> are similar; perhaps, like PfSEA-1 and MSP7, this protein is also required during merozoite egress from schizonts and/or subsequent invasion into fresh erythrocytes.

**Expression of the three candidates identified by Raj et al. <sup>1)</sup> in *P. falciparum* blood stages, from data available on PlasmoDB**



Data from PlasmoDB:  
Aurecochea C, et al. 2008. **PlasmoDB: a functional genomic database for malaria parasites.** *Nucleic Acids Res.* 37: D539-43.

*P. falciparum* 3D7 transcriptome data:  
Bozdech Z, Linás M, Pulliam BL, et al. 2003. **The Transcriptome of the Intraerythrocytic Developmental Cycle of *Plasmodium falciparum*.** *PLoS Biol.* 1: e5.

Protein expression data sources not yet available from PlasmoDB.

In future, it is hoped that PfSEA-1 will add to our arsenal of malaria vaccine candidates, and that further candidates could be similarly identified, by comparing plasma or cellular recognition of antigens in patient cohorts that experience differing risks of severe malaria or even infection with other *Plasmodium* species.

## References

1. Raj DK, Nixon CP, Nixon CE et al. 2014. **Antibodies to PfSEA-1 block parasite egress from RBCs and protect against malaria infection.** *Science.* 344: 871.
2. Yeoh S, O'Donnell RA, Koussis K, et al. 2007. **Subcellular discharge of a serine protease mediates release of invasive malaria parasites from host erythrocytes.** *Cell.* 131:1072.
3. Kadekoppala M, O'Donnell RA, Grainger M, et al. 2008. **Deletion of the *Plasmodium falciparum* merozoite surface protein 7 gene impairs parasite invasion of erythrocytes.** *Eukaryot Cell.* 7: 2123.
4. Tewari R, Ogun SA, Gunaratne RS, et al. 2005. **Disruption of *Plasmodium berghei* merozoite surface protein 7 gene modulates parasite growth *in vivo*.** *Blood.* 105: 394.
5. Spaccapelo R, Aime E, Caterbi S, et al. 2011. **Disruption of plasmepsin-4 and merozoites surface protein-7 genes in *Plasmodium berghei* induces combined virulence-attenuated phenotype.** *Scientific Reports* 1: 39.