A directed-evolution approach to reveal the immunoglobulin M binding site of Duffy-binding-like domains in malarial parasite-encoded proteins.

Shona Moore[1,2], Pat Blundell[1], Dan Czajkowsky[3] and Richard Pleass[1]

[1]Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA, UK; [2]Warwick Systems Biology Centre, University of Warwick, Coventry, CV4 7AL, UK; [3]Shanghai Institute of Applied Physics, Chinese Academy of Sciences.

Background

Duffy-binding-like (DBL) protein domains are essential to the function of malarial proteins

Proteins expressed by the malaria parasite are involved in a range of immune evasion mechanisms [1]. One such family of proteins, PfEMP1, plays an important role in antigenic variation and interacts with hundreds of host ligands, leading to diverse disease outcomes. DBL domains are essential to the function of these proteins. However, it is unclear how the DBL domains facilitate host-receptor interactions.

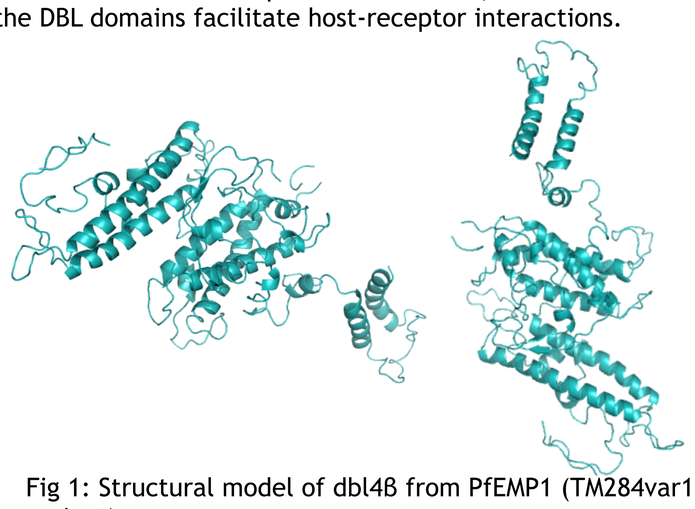

Fig 1: Structural model of dbl4ß from PfEMP1 (TM284var1 variant).

Non-specific IgM binds to the Duffy-binding-like (DBL) malarial protein domain and increases parasite survival

At least ten DBL domains from members of the PfEMP1 family have been shown to bind non-specific IgM [2]. The sequence motif underpinning IgM binding is currently unknown.

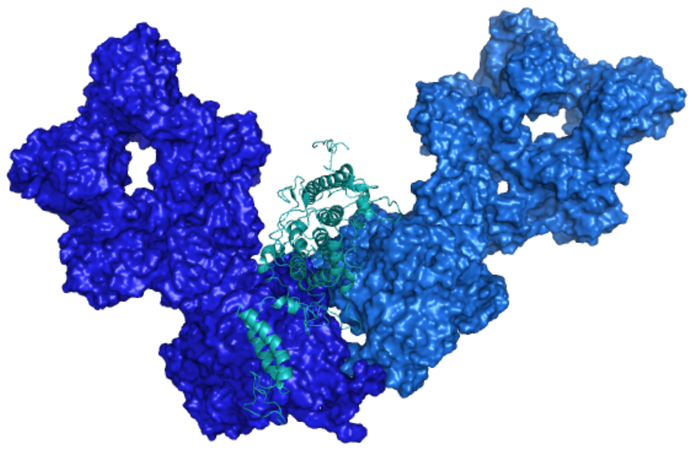

Fig 2: Binding of Dbl4ß (cyan) to IgM. The DBL domain binds to the cμ4 domain on the Fc region of IgM and docks between two monomers of IgM (navy and blue).

Possible functional consequences of the IgM-DBL interaction

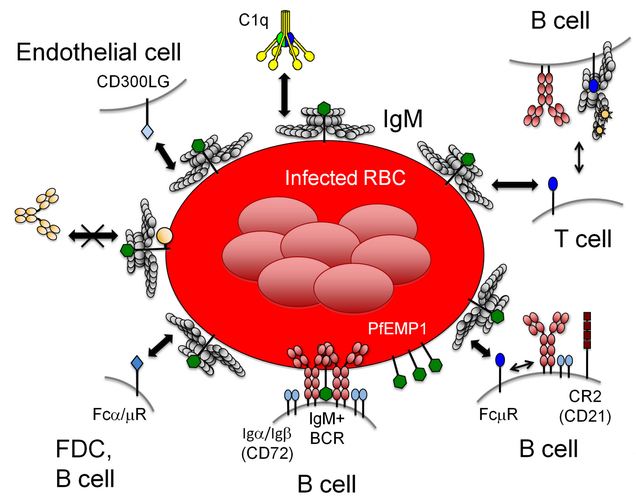

Fig 3: Possible functional consequences of the PfEMP1–IgM interaction include interference with immunological signaling and clearance mechanisms, blocking the binding of specific Abs or a way of enhancing infected erythrocyte sequestration [3].

Aims

1. Express wild type DBL domains.
2. Create a library of mutants.
3. Calculate binding affinities for mutants.
4. Map mutations onto structural model.
5. Identify residues critical for IgM binding.

Results

1. DBL-Fc fusion proteins have been cloned and IgM binding confirmed using enzyme-linked immunosorbent assay (ELISA).

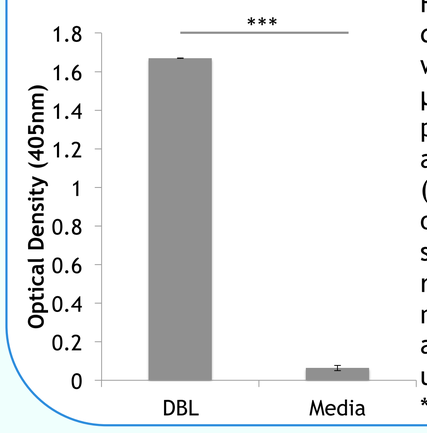

Fig 4: A sandwich ELISA to detect DBL-IgM binding. IgM was coated on the plate at 5 μg/ml. DBL-Fc purified by protein G column was used at 175 μg/mL and αhIgG1-Fc (AP) was used at a dilution of 1:5000. PNPP was used as substrate, and the plate was read at 405 nm. Data are means ± s.e.m. Statistical analysis was by a two-tailed unpaired Student's t test; ***P < 0.001.

2. A library of ~150 DBL mutants has been created by error-prone PCR and mutants selected for expression based on structural information.

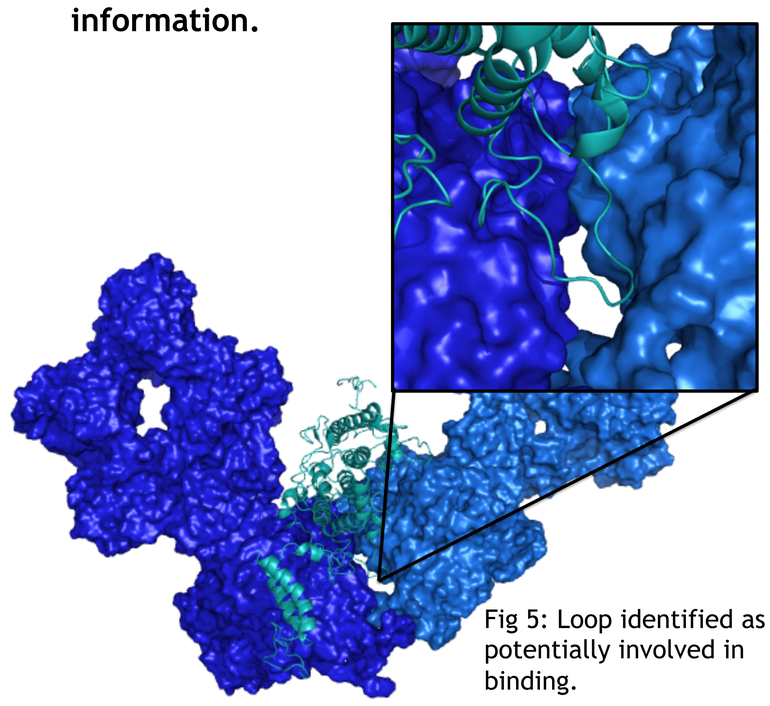

Fig 5: Loop identified as potentially involved in binding.

Residues potentially involved in IgM binding were identified using the structural model and mutants produced by random mutagenesis were selected for transfection based on this information.

3. DBL-Fc mutants were expressed in ChoK1 cells and their affinity of binding to IgM investigated by ELISA and SPR.

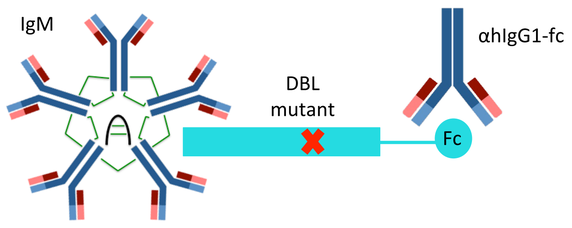

Fig 6: Schematic of DBL-IgM binding assay.

4. Mutations were mapped onto structural model.

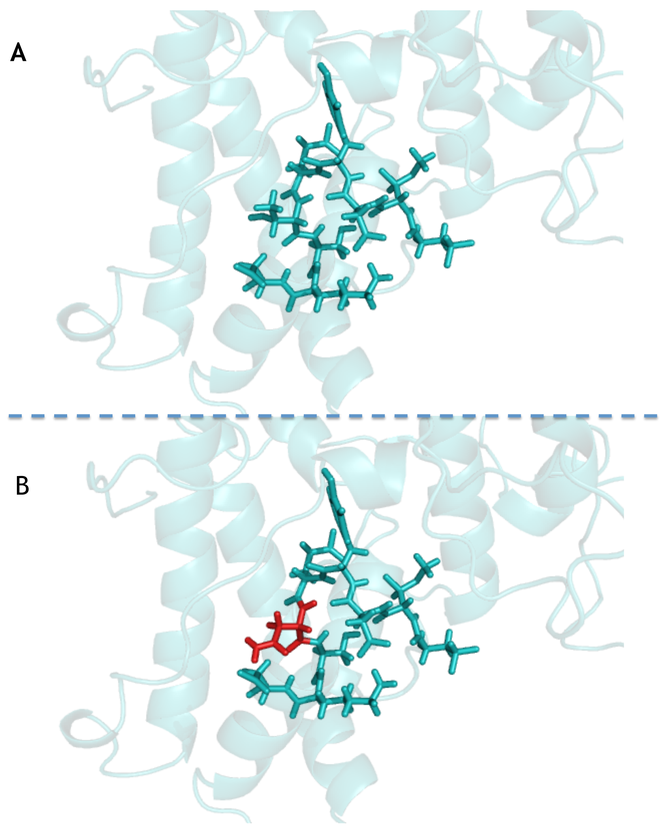

Fig 7: (A) Wild type DBL domain vs (B) mutant DBL domain with threonine mutated to asparagine, a mutation which may impact on binding.

5. Amino acids outside of the proposed IgM binding site are critical for IgM-binding.

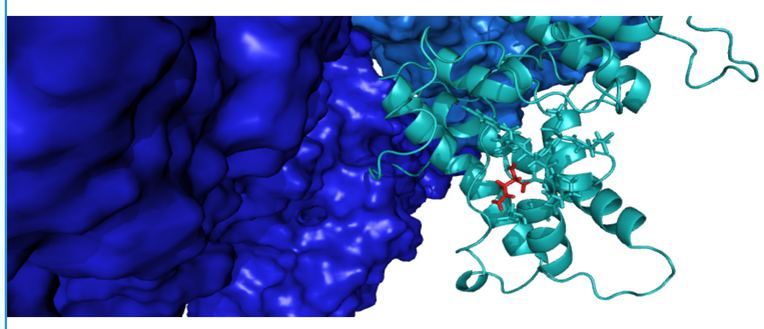

Fig 8: DBL mutant with threonine to asparagine mutation mapped onto structural model and compared to proposed IgM binding region.

Discussion

The structural model may either be inaccurate, or amino acids outside the proposed interaction site also affect binding (framework mutations). DBL mutant data will be used to improve on the current model and identify further critical residues. We hope to fully characterise the IgM-binding site of DBL domains using this updated structural model.

References

[1] The International Journal of Biochemistry & Cell Biology, 2005 vol. 37 (4), pp. 700-706.
[2] Journal of Immunology, 2010 vol. 184 (9) pp. 4597-603.
[3] Proceedings of the National Academy of Sciences of the United States of America, 2011 vol. 108 (30) pp. 12485-90.

Acknowledgements

We wish to acknowledge the British Society for Parasitology for their support which made it possible to attend this meeting.

BRITISH SOCIETY FOR PARASITOLOGY BSP

THE UNIVERSITY OF WARWICK

EPSRC Engineering and Physical Sciences Research Council

LSTM LIVERPOOL SCHOOL OF TROPICAL MEDICINE