Applying recombinant protein technology to study *Plasmodium falciparum* erythrocyte receptor-ligand interactions and their potential as therapeutic targets

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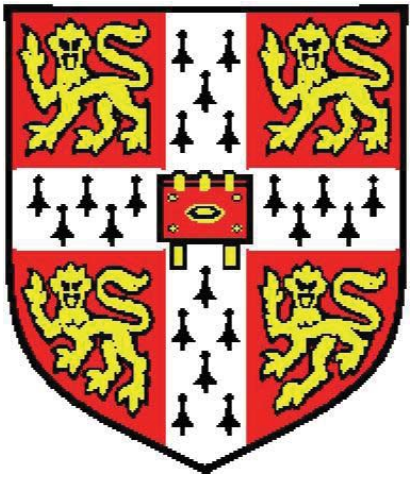

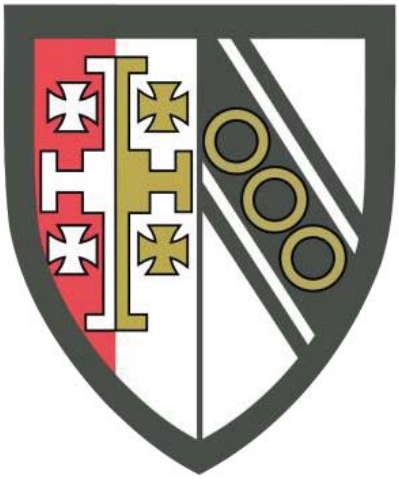

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This dissertation is submitted for the degree of Doctor of Philosophy

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This PhD thesis is dedicated to

Anna,

my dearest friend who passed

away in November 2012...

DECLARATION

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text.

This thesis does not exceed the word limit of 60,000 as set by the Degree Committee for the Faculty of Biology.

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ABSTRACT

Plasmodium falciparum is the causative agent of the most severe form of malaria, a serious infection accounting for an estimated one million deaths annually. Currently, there is no licenced malaria vaccine available. Erythrocyte invasion by the blood stage of the parasite, the merozoite, is a critical step in parasite's lifecycle and has long been targeted for the development of therapeutics. However, the development of a highly efficacious blood stage malaria vaccine has been challenged by the highly polymorphic nature of merozoite ligands, as well as by functional redundancy of the receptor-ligand pairs involved in the invasion process. The identification of cell surface receptor-ligand interactions which are essential for erythrocyte invasion by merozoite has been hampered by the difficulties in recombinant expression of *Plasmodium* spp. proteins, and by the technical challenges in systematically identifying interacting partners for cell surface proteins.

In earlier work from our laboratory, BSG and Semaphorin7A were identified as the erythrocyte receptors for merozoite RH5 and MTRAP, respectively. Importantly, the interaction between RH5 and BSG is the only one known so far that is essential and universally required for erythrocyte invasion. Antibodies against either RH5 or BSG could potently inhibit erythrocyte invasion in all *P. falciparum* strains tested; therefore, the blockade of RH5-BSG interaction can be exploited for therapeutic purposes, for the treatment of *P. falciparum* infected individuals.

Most of the work described in this thesis is aimed at the development of a humanised or chimeric anti-BSG antibody as a potential anti-malarial therapeutic. For this purpose, a plasmid system, which enables the recombinant expression of engineered antibodies, was established. By using this versatile plasmid system, two anti-BSG monoclonals, MEM-M6/4 and MEM-M6/8 (obtained from a published report), were successfully humanised by Complementarity Determining Region (CDR) grafting. The therapeutic potential of huMEM6-M/4 and huMEM6-M/8 was hindered by the low affinity of these antibodies for BSG. To obtain a higher potency antibody, a panel of hybridoma lines secreting anti-BSG antibodies was generated by directly immunising animals. A monoclonal antibody, m6D9, secreted by one of the generated hybridoma clones, demonstrated high efficacy in inhibiting erythrocyte invasion in parasite culture, and was selected for chimerisation. The chimeric antibody, ch6D9, retained its high affinity for BSG and blocked erythrocyte invasion

at very low concentrations in all parasite lines tested. Furthermore, ch6D9 displayed reduced binding to FcγRIIA and C1q *in vitro*, suggesting that this antibody may have reduced ability to trigger antibody effector functions. Attempts to fully humanise this antibody were unsuccessful.

In another approach, two anti-RH5 monoclonals (2AC7 and 9AD4; Douglas *et al.*, 2013) were successfully chimerised. Both ch2AC7 and ch9AD4 preserved their high affinity for RH5 and inhibited erythrocyte invasion in parasite culture, but with much higher IC_{50} as compared to ch6D9. The variable regions of ch2AC7 and ch6D9 were combined in an anti-RH5 and anti-BSG bi-specific antibody: 2AC7-6D9 DVD-Ig. 2AC7-6D9 DVD-Ig was capable of simultaneous binding to RH5 and BSG but its affinity for BSG decreased in comparison to ch6D9. When tested in a *P. falciparum* growth inhibition assay, 2AC7-6D9 DVD-Ig was more efficient than ch2AC7, but less than ch6D9 in blocking erythrocyte invasion.

Finally, in a parallel project, I aimed to identify novel receptor-ligand pairs involved in erythrocyte invasion. For this purpose, I expanded an existing *P. falciparum* merozoite recombinant protein library by 26 proteins, which were chosen based on transcription microarray data, and information available in the literature. The new members of the *P. falciparum* protein library were recombinantly expressed and systematically screened against an equivalent library consisting of erythrocyte receptors. The screen identified a putative interaction (PF13_0125 – P4HB). Further characterisation of the identified interaction provided inconclusive results, and more experiments are required to confirm its validity. The recombinant *P. falciparum* merozoite proteins reported in this project should prove to be a useful tool for the deeper understanding of erythrocyte invasion, and *P. falciparum* biology in general.

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