Karolinska Institutet

Institutionen för Mikrobiologi, Tumör och Cell Biologi

Exported proteins of the malaria parasite *Plasmodium falciparum* – Characterization of blood-stage antigen 332

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ABSTRACT

Plasmodium falciparum malaria is one of the most important infectious diseases in the world. Following invasion of the human red blood cell (RBC), the *P. falciparum* parasite dramatically remodels its host cell by introducing a parasite-derived trafficking machinery in RBC cytosol, interacting with the submembrane skeleton and expressing adhesins on the RBC surface. All host cell modifications are mediated by a subset of parasite-encoded proteins, which are exported beyond the confines of the parasite – a feature that is fundamental to the malaria pathogenesis. Central to this thesis is the Pf332 protein, the largest protein exported into the host cell cytosol. Although identified more than two decades ago, the function of Pf332 still remains elusive. Regardless, the location of Pf332 in close proximity to the RBC plasma membrane, its potential surface expression, characteristic protein structure and immunogenic nature make it an important antigen to study. We have revised the structure of the gene encoding Pf332, and identified a previously unknown first exon encoding an RBC-binding Duffy binding-like (DBL)-domain homologous to DBL-domains present in a family of invasion proteins. Studies on Pf332 have been hampered by the cross-reactive nature of antibodies generated against the molecule due to its high content of glutamic acid-rich repeats. In an attempt to evaluate the potential of the DBL-domain as a specific marker for Pf332, we set out to analyze the tertiary structure of the domain and the specificity of naturally acquired antibodies. Although the predicted structure of the DBL-domain was similar to that of the homologous domains present in invasion proteins, acquired antibodies were specific for Pf332. Thus, the DBL-domain can be used as a specific Pf332 marker and we expect this to facilitate further investigations of the antigen. Subunit vaccines based on recombinant proteins are often hampered by low antigenicity, thus adjuvants are of major importance. We set out to study the immunogenicity of a recombinant Pf332 DBL-domain in combination with adjuvants compatible for human use, in rodents and rabbits. The domain was found to be immunogenic and of the three adjuvants evaluated, Montanide ISA 720 appeared to be the most suitable adjuvant, as it induced a more long-lasting Th2-biased antibody response. Thus, the results support the use of Montanide ISA 720 for future immunization studies of other malaria vaccine candidates. To investigate the subcellular location and the solubility characteristic of Pf332, we employed a biochemical approach in combination with immunofluorescence microscopy. We found Pf332 to be a host cytoskeleton interacting protein that is synthesized as a peripheral membrane protein and associates with the cytosolic side of Maurer's clefts via protein-protein interactions throughout trophozoite maturation and schizogony. Importantly, our data show that Pf332 is not expressed on the surface of the host cell, but may have important functions in host cytoskeleton remodeling at the end of the intraerythrocytic developmental cycle. The gene encoding Pf332 is duplicated in the HB3 parasite, having only slight sequence variation between the two gene copies. This enabled us to develop a sensitive allelic discriminative assay, which can be used to study transcriptional activity of duplicated genes in the *P. falciparum* genome. We employed the assay to study the maternal malaria associated *var* gene *var2csa*, which is similarly found duplicated in the HB3 parasite. Both *var2csa* paralogs were simultaneously transcribed in a single cell, thus contradicting the mutually exclusive expression of *var* genes in *P. falciparum*. In conclusion, by using Pf332 as a model protein for studying malaria pathogenesis, we have not only obtained novel information regarding the protein itself, but gained important knowledge and developed versatile techniques, which can be used to study a wide array of other malaria antigens.

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