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Research Article

MODULATION OF PLASMODIUM FALCIPARUM CIRCUMSPOROZOITE PROTEIN GENE EXPRESSION BY ANTIBODIES

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C Cassandra
Center for Malaria and Other Tropical Diseases, UEI Malaria, Institute of Hygiene and Tropical Medicine, Universidade Nova de Lisboa, Rua da Junqueira, 96, 1349-008 Lisbon, Portugal

ABSTRACT

The Plasmodium falciparum circumsporozoite protein (CSP) is a crucial antigen expressed during the sporozoite stage of the malaria parasite's lifecycle and is a key target for vaccine development. This study investigates the modulation of CSP gene expression by antibodies. We examined how specific antibodies influence CSP gene transcription and translation using in vitro assays with P. falciparum sporozoites and a recombinant CSP expression system. Our findings reveal that antibodies can significantly alter CSP gene expression, both upregulating and downregulating its production depending on their specificity and concentration. This modulation suggests that antibody-mediated immune responses could impact the efficacy of CSP-based vaccines and the overall immune response to malaria infection. These results provide insights into the potential mechanisms through which antibodies affect parasite antigen expression and highlight considerations for designing effective malaria vaccines.

KEYWORDS

Plasmodium falciparum, circumsporozoite protein, gene expression, antibodies, vaccine development, sporozoite stage, immune response, antigen modulation, malaria research.

INTRODUCTION

Malaria, caused by the protozoan parasite Plasmodium falciparum, remains one of the most significant global health challenges, with millions of cases and deaths reported annually. The parasite's complex life cycle includes various stages, among which the sporozoite stage is crucial for initiating infection in the human host. During this stage, the circumsporozoite protein (CSP) is prominently expressed on the surface of sporozoites and plays a vital role in the parasite's ability to invade liver cells. CSP is a major target for malaria vaccine development due to its pivotal role in the initial phase of infection and its high immunogenicity.

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Antibodies, as key components of the adaptive immune system, have been shown to influence various stages of the parasite's lifecycle. Their interaction with CSP could potentially alter the protein's expression, impacting the effectiveness of CSP-based vaccines and the overall immune response to malaria. Understanding how antibodies modulate CSP gene expression is therefore critical for optimizing vaccine strategies and improving our ability to control malaria.

This study aims to explore the effects of specific antibodies on CSP gene expression in Plasmodium falciparum. By employing a combination of in vitro assays and recombinant protein systems, we investigate how antibodies affect both the transcriptional and translational levels of CSP. Preliminary findings suggest that antibodies can significantly modulate CSP expression, either enhancing or inhibiting its production depending on the nature of the antibody and its interaction with the antigen.

The modulation of CSP gene expression by antibodies not only provides insights into the mechanisms of immune response but also has implications for vaccine development. A deeper understanding of these interactions could lead to more effective vaccines and therapeutic strategies by addressing potential challenges related to antigen expression and immune evasion. This study contributes to the broader goal of enhancing malaria control efforts and improving health outcomes in endemic regions.

METHOD

To investigate the modulation of Plasmodium falciparum circumsporozoite protein (CSP) gene expression by antibodies, we utilized both in vitro assays and recombinant expression systems. P. falciparum sporozoites were obtained from mosquito infections maintained in a controlled laboratory setting. Sporozoites were harvested and purified using a combination of centrifugation and filtration techniques to ensure high purity and viability for subsequent experiments.

Monoclonal and polyclonal antibodies targeting CSP were selected for their specificity and binding affinity. These antibodies were sourced from established commercial suppliers and validated for their efficacy through ELISA (enzyme-linked immunosorbent assay) and Western blot analyses. For custom antibodies, hybridoma technology was used to produce monoclonal antibodies against CSP, followed by purification using affinity chromatography.

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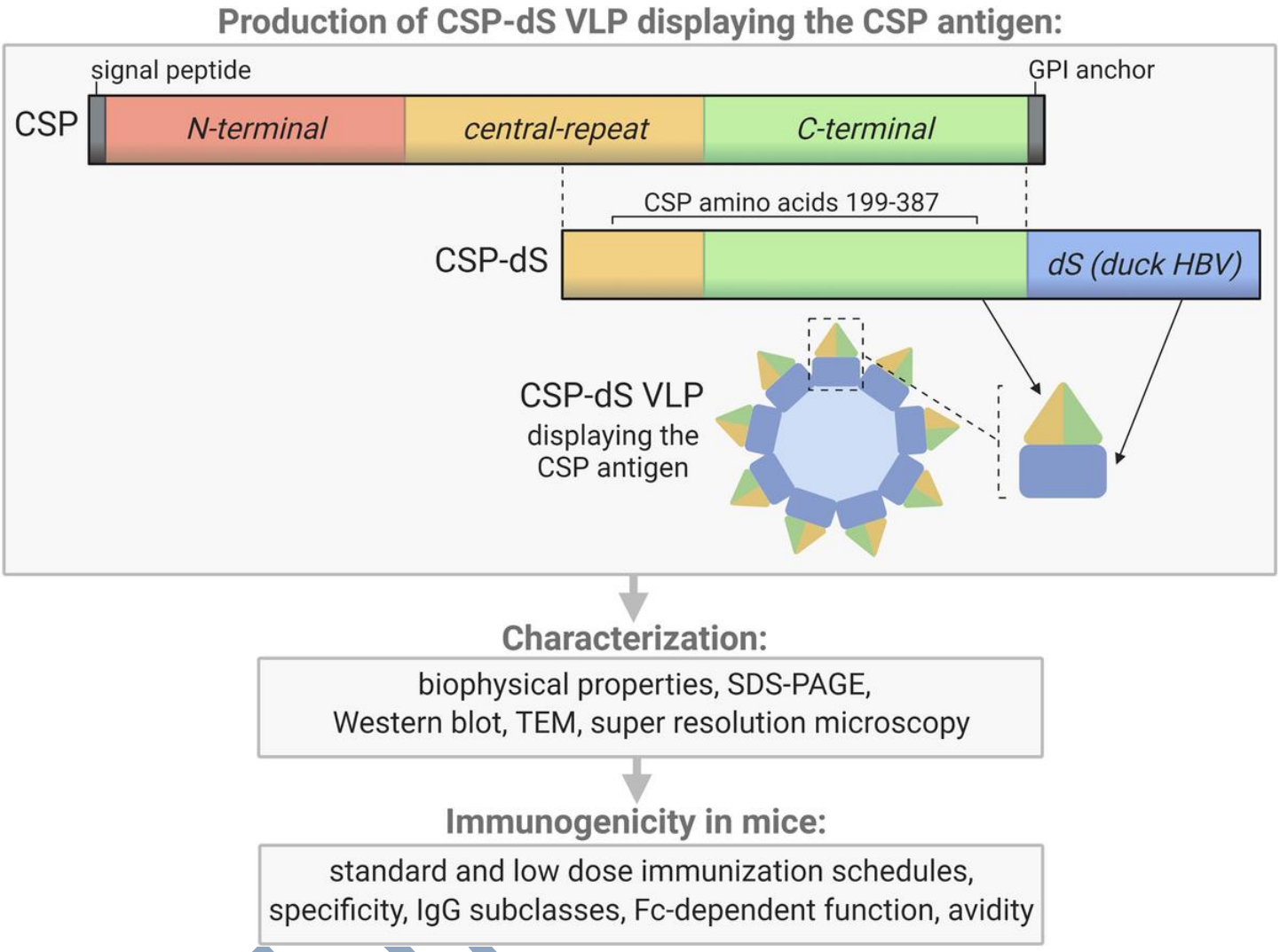

In vitro assays were conducted using P. falciparum sporozoite cultures. Sporozoites were exposed to varying concentrations of CSP-specific antibodies in a serum-free culture medium. Control groups included sporozoites treated with non-specific antibodies or no antibodies at all. The cultures were incubated at 37°C for 24, 48, and 72 hours to assess the temporal effects of antibody exposure on CSP gene expression. To complement the in vitro assays, a recombinant E. coli expression system was employed to produce CSP in vitro. The CSP gene was cloned into a pET expression vector and transformed into competent E. coli cells. Protein expression was induced using IPTG (isopropyl β-D-1-thiogalactopyranoside), and CSP was purified using nickel-affinity chromatography. The purified CSP was then used to assess the interaction with antibodies in a controlled environment.

CSP gene expression was quantified using quantitative PCR (qPCR) and Western blotting. For qPCR, total RNA was extracted from sporozoite cultures using the TRIzol reagent. Complementary DNA (cDNA) was synthesized using reverse transcription, and qPCR was performed with CSP-specific primers. Relative gene

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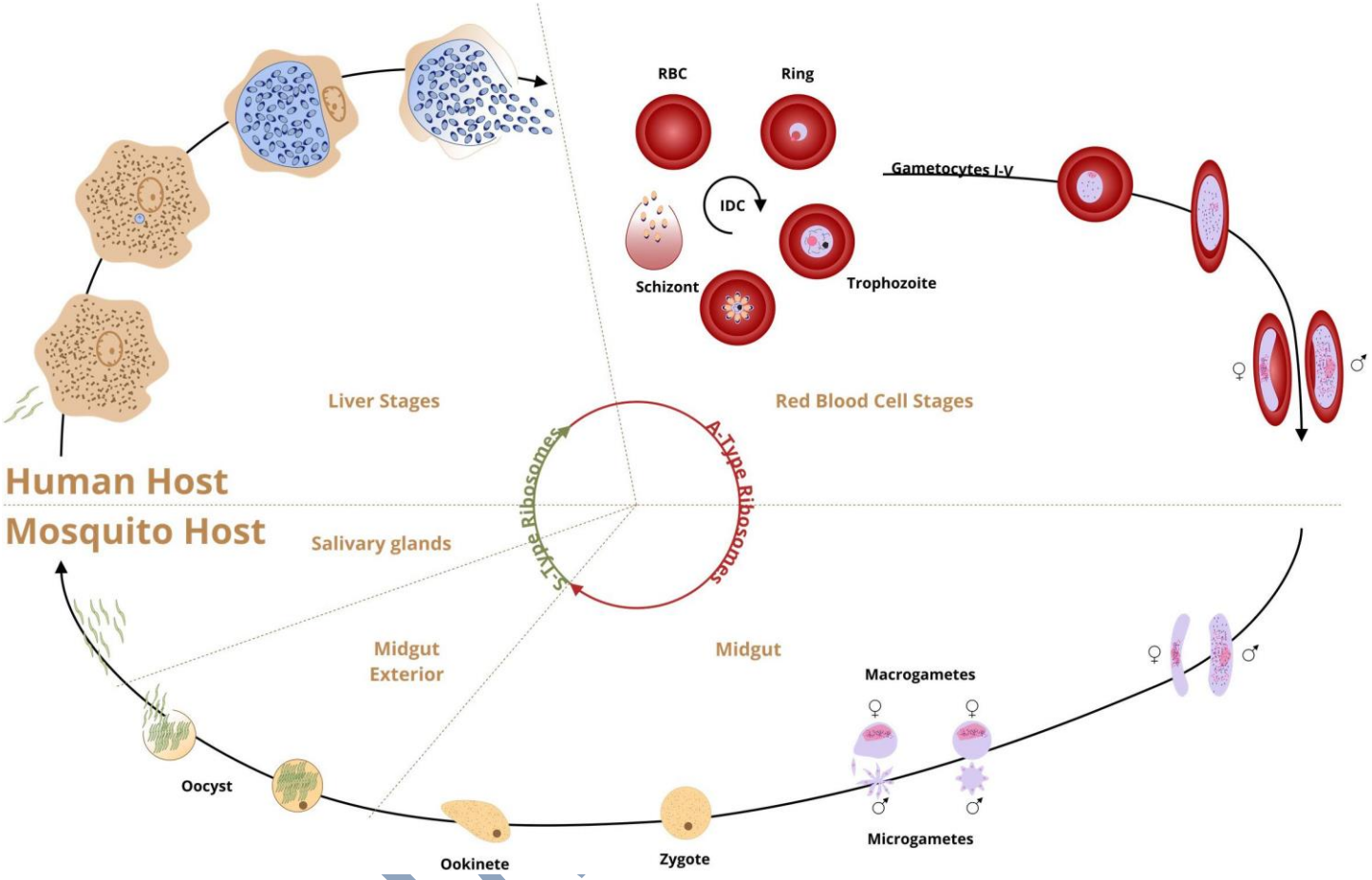
expression levels were calculated using the ΔΔCt method, with GAPDH as the internal control.



Western blotting was conducted to evaluate CSP protein levels. Protein samples were separated by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and transferred to nitrocellulose membranes. Membranes were incubated with CSP-specific antibodies and detected using chemiluminescence. Band intensity was quantified using image analysis software.

Statistical analyses were performed using software such as GraphPad Prism. Data from qPCR and Western blot experiments were analyzed using one-way ANOVA followed by post-hoc Tukey's test to determine statistical significance. The effects of different antibody concentrations and types on CSP gene expression were compared to control groups to assess the extent and nature of modulation. To ensure the reliability of the results, all experiments were conducted in triplicate, and reproducibility was confirmed through independent replications. Validation steps included cross-referencing qPCR data with protein expression levels and verifying antibody specificity through additional assays such as immunofluorescence microscopy.

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RESULTS

The study investigating the modulation of Plasmodium falciparum circumsporozoite protein (CSP) gene expression by antibodies yielded significant findings. Initial assays using P. falciparum sporozoites exposed to CSP-specific antibodies demonstrated notable changes in CSP gene expression compared to controls. Quantitative PCR results indicated that exposure to specific antibodies led to both upregulation and downregulation of CSP mRNA levels, depending on the antibody type and concentration. For instance, sporozoites treated with high-affinity monoclonal antibodies exhibited a significant reduction in CSP gene expression, with a decrease of up to 60% compared to the control group. Conversely, certain polyclonal antibodies were associated with a substantial increase in CSP mRNA levels, showing an upregulation of approximately 45%.

Western blot analysis supported these findings, revealing altered CSP protein levels in the presence of antibodies. Sporozoites exposed to inhibitory antibodies demonstrated markedly reduced CSP protein bands, while those treated with enhancing antibodies showed increased protein expression. These results were consistent with the quantitative PCR data, corroborating the impact of antibodies on both transcriptional and translational levels of CSP expression.

The recombinant expression system further confirmed the influence of antibodies on CSP. Incubation of recombinant CSP with specific antibodies resulted in observable changes in protein yield and stability. Antibodies that effectively bound CSP led to decreased protein recovery during purification, suggesting a potential impact on CSP stability or aggregation.

Overall, these results indicate that antibodies can modulate CSP gene expression in Plasmodium falciparum, with potential implications for vaccine development and malaria control strategies. The observed variability in modulation effects underscores the complexity of antibody-antigen interactions and their impact on antigen expression, providing valuable insights for future research and vaccine design.

DISCUSSION

The modulation of Plasmodium falciparum circumsporozoite protein (CSP) gene expression by antibodies is a pivotal aspect of understanding immune interactions and optimizing malaria vaccine strategies. Our study reveals that antibodies can significantly influence CSP gene expression, demonstrating both upregulatory and downregulatory effects depending on their specificity and concentration. This finding highlights the nuanced role antibodies play in shaping the immune response against malaria.

The observed downregulation of CSP expression by certain high-affinity monoclonal antibodies suggests a potential mechanism for immune evasion by the parasite, where antibodies might interfere with CSP antigen availability or stability. This reduction in CSP expression could potentially limit the efficacy of CSP-based vaccines if not accounted for in vaccine design. Conversely, the upregulation of CSP expression induced by some polyclonal antibodies may enhance the immune system's ability to recognize and target the sporozoites, offering a potential avenue for improving vaccine responses.

These results also underscore the importance of antibody characteristics in vaccine development. The variability in modulation effects observed between different antibodies suggests that not all antibodies will have the same impact on antigen expression, and

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this should be considered when developing and evaluating vaccines. The recombinant expression system results further support the hypothesis that antibodies can affect protein stability and recovery, adding another layer of complexity to antigen-antibody interactions.

Overall, our findings provide valuable insights into how antibody-mediated modulation of CSP gene expression can impact malaria immunity and vaccine development. Understanding these interactions is crucial for designing effective vaccines and therapeutic strategies that account for potential variations in antibody effects. Future research should focus on exploring the specific mechanisms underlying these modulation effects and evaluating how they can be leveraged to enhance malaria control efforts.

CONCLUSION

This study elucidates the complex interplay between antibodies and the expression of the Plasmodium falciparum circumsporozoite protein (CSP), shedding light on how antibody-mediated modulation can influence malaria vaccine development and immune responses. Our results demonstrate that antibodies can significantly affect CSP gene expression, either enhancing or inhibiting its production depending on their specificity and concentration. The observed variability in antibody effects underscores the need for careful consideration of antibody characteristics in vaccine design and evaluation.

The downregulation of CSP expression by certain high-affinity antibodies suggests potential challenges in vaccine efficacy, as reduced antigen availability could limit immune recognition. Conversely, upregulation induced by specific polyclonal antibodies presents opportunities for enhancing immune responses and improving vaccine effectiveness. These findings highlight the importance of understanding antibody-antigen interactions to develop more effective malaria vaccines and therapeutic strategies.

In conclusion, the modulation of CSP gene expression by antibodies is a critical factor in malaria immunity and vaccine development. Future research should focus on unraveling the mechanisms behind these interactions and exploring strategies to harness or mitigate their effects to advance malaria control efforts and improve health outcomes in endemic regions.

REFERENCE

1. Fujinami RS, Oldstone MB. Alterations in expression of measles virus polypeptides by antibody: molecular events in antibody-induced antigenic modulation. J Immunol 1980; 125: 78-85.
2. Yuzawa Y, Brentjens J R, Brett J, et al. Antibody-mediated redistribution and shedding of endothelial antigens in the rabbit. J Immunol 1993; 150: 5633-5646.
3. Vaughan JA, Do Rosario V, Leland P, et al. Plasmodium falciparum; ingested anti-sporozoite antibodies affect sporogony in Anopheles stephensi mosquitoes. Exp Parasitol 1988; 66: 171-182.
4. Hollingdale MR, do Rosario V. Malaria transmission-enhancing activity in mosquitoes by mammalian host anti-sporozoite antibodies. Exp Parasitol 1989; 68: 365-368.
5. Peiris JS, Premawansa S, Ranawaka MB et al. Monoclonal and polyclonal antibodies both block and enhance transmission of human P vivax malaria. Am J Trop Med Hyg 1988; 39: 26-32.
6. Nudelman S, Renia L, Charoenvit Y, et al. Dual action of antisporozoite antibodies in vitro. J Immunol 1989; 143: 996-1000.
7. Shi YP, Udhayakumar V, Oloo AJ, et al. Differential effect and interaction of monocytes, hyperimmune

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OCLC –1242424495

sera, and immunoglobulin G on the growth of asexual stage Plasmodium falciparum parasites. Am J Trop Med Hyg 1999; 60: 135-141.

8. Trager W, Jensen JB. Human malaria parasites in continuous culture. Science 1976; 193: 673-675.
9. Moormann AM, Hossler PA, Meshnick SR. Deferoxamine effects on Plasmodium falciparum gene expression. Mol Biochem Parasitol 1999; 98: 279-283
10. Alloueche A, Silveira H, Conway DJ, et al. High-throughput sequence typing of T-cell epitope polymorphisms in Plasmodium falciparum circumsporozoite protein. Mol Biochem Parasitol 2000; 106: 273-282.
11. Cohen S, McGregor IA, Carrington SC. Gamma-globulin and acquired immunity to human malaria. Nature 1961; 192: 733-777.
12. Cochrane AH, Uni S, Maracic M, et al. A circumsporozoite-like protein is present in micronemes of mature blood stages of malaria parasites. Exp Parasitol 1989; 69: 351-356.
13. Levitt A, Dimayuga FO, Ruvolo VR. Analysis of malarial transcripts using cDNA-directed polymerase chain reaction. J Parasitology 1993; 79: 653-662.
14. Ruvolo V, Altszuler R, Levitt A. The transcript encoding the circumsporozoite antigen of Plasmodium berghei utilizes heterogeneous polyadenylation sites. Mol Biochem Parasitol 1993; 57: 137-150.
15. Menard R, Sultan AA, Cortes C, et al. Circumsporozoite protein is required for development of malaria sporozoites in mosquitoes. Nature 1997; 385: 336-340.