Development and characterization of murine monoclonal antibodies against allelic variants of the *Plasmodium vivax* circumsporozoite protein (VK210, VK247, and *P. vivax*-like) and its C-terminal region

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Malaria caused by *Plasmodium vivax* is widely distributed and represents a significant challenge for disease control. The circumsporozoite protein (CSP) is essential for hepatocyte invasion and is therefore considered an important target for the development of vaccines and neutralizing antibodies. However, the presence of different allelic variants of *Pv*CSP (VK210, VK247, and *P. vivax*-like) represents an additional challenge for intervention strategies. In this context, the aim of this study was to produce and characterize murine monoclonal antibodies (mAbs) against the allelic variants and the C-terminal region of *Pv*CSP. For this purpose, C57BL/6 mice were immunized with recombinant chimeric versions of the three allelic variants of *Pv*CSP, followed by hybridoma generation, selection of responsive clones by ELISA, and characterization of the mAbs produced by ELISA and Western blot. Five mAbs were obtained, two targeting the C-terminal region of *Pv*CSP and one specific for each of the three allelic variants. Immunological characterization demonstrated that the antibodies predominantly belonged to the IgG1, IgG2b, and IgG2c subclasses, all presenting kappa-type light chains. Specificity assays showed selective recognition of the corresponding variants, with no cross-reactivity. Furthermore, in immunofluorescence assays the mAbs were able to recognize *P. vivax* sporozoites, and one of them showed the potential to block hepatocyte invasion and to induce death in *P. vivax* sporozoites in flow cytometry assays. Together, these results indicate that the antibodies generated constitute promising tools for the study of *P. vivax* malaria and may provide insights for future vaccine approaches against the disease.

Keywords: *Plasmodium vivax*; Monoclonal antibodies; Circumsporozoite protein; Allelic variants; Hybridoma; Malaria.

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