SUMMARY
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Merozoite Surface Protein-9 is one of the earliest merozoite proteins identified as a vaccine candidate in *Plasmodium falciparum*, however its potential to induce protective immune response in animal models has not been assessed until now. The main objective of this work was to evaluate the protective efficacy of the immunization with Merozoite Surface Protein-9 from a rodent malaria parasite, in a mouse model for homologous parasite challenge. Another objective of this study was the biochemical and immunological characterization of recombinant MSP-9 from *P. berghei* and its comparison with the published information about other MSP-9 proteins, in particular *P. falciparum* MSP-9 (PfMSP-9).

A combination of degenerate PCR and RACE was used to amplify *P. berghei* msp-9 gene (*Pbmsp*-9) from genomic DNA. *P. yoelii* msp-9 gene (*Pymsp*-9) was identified from a BLASTX analysis using PfMSP-9 nucleotide sequence as a query and the gene was amplified from *P. yoelii* genomic DNA using specific primers. Comparison of these two genes (*Pbmsp*-9 and *Pymsp*-9) with other members of the MSP-9 family, demonstrated that they contain typical features of this protein family like the presence of 4 conserved cysteines at the N-terminal region and the SGG motif that has been associated with the putative protease activity of PfMSP-9. However, the tandem repeat region at the C-terminal end, characteristic of all the MSP-9 proteins, is present in *P. yoelii* MSP-9 but not in *P. berghei* MSP-9 (Figure 3.6 A).

To carry out immunological and biochemical studies, recombinant constructs representing different N-terminal fragments of *P. berghei* MSP-9 protein were prepared. Expression of the recombinant proteins was carried out in the *E. coli* system, however all the proteins were produced as insoluble material in inclusion bodies. As has been reported for *P. falciparum* MSP-9, larger recombinant proteins representing the N-terminal region of PbMSP-9 were highly unstable and difficult to purify. Different methods of purification of the insoluble proteins were tried. A method of refolding by rapid dilution followed by ion-exchange chromatography provided a reasonable yield of pure recombinant proteins.
PbMSP-9 (NM) recombinant protein (aa 23 – 370) was selected to carry out the immunogenicity and challenge studies, because it represents the largest N-terminal region of PbMSP-9 that was successfully expressed, and it contains the conserved regions that have been implicated in the putative functions of MSP-9 homologues in the parasite.

The following are the salient features of the present study:

- The genes of Merozoite Surface Protein 9 from *P. berghei* and *P. yoelii* were identified and characterized.

- The presence of PbMSP-9 and PyMSP-9 transcripts was demonstrated in *P. berghei* and *P. yoelii* parasites by northern blot analyses.

- The expression and the localization of the native proteins in the merozoites were confirmed by Western blot analysis of *P. yoelii* and *P. berghei* parasite lysate and immunofluorescence assay.

- Different recombinant fragments of PbMSP-9 were cloned and expressed in *E. coli*. Purification and refolding of the recombinant proteins corresponding to different N-terminal fragments [PbMSP-9 (Ncys), PbMSP-9 (N), PbMSP-9 (NM)] was carried out.

- Recombinant PbMSP-9 (NM) showed chymotrysin-like activity against the fluorogenic substrate NSuc-LLVY-AMC that was inhibited by the serine protease inhibitors chymostatin, PMSF and leupeptin. These results suggest that the recombinant protein PbMSP-9 (NM) was correctly folded and that native PbMSP-9 could be a chymotrysin-like protease in *P. berghei* parasites. In addition, the three recombinant fragments representing different lengths of the N-terminal region of PbMSP-9 were able to bind to mouse erythrocytes.
• The immunogenicity of PbMSP-9 (NM) formulated in Alum or in CFA was evaluated in two strains of mice with different genetic background (BALB/c and C56BL/6).

• PbMSP-9 (NM) recombinant protein was found to be immunogenic in both strains of mice, with maximum antibody titers observed in the BALB/c group immunized with protein formulated in CFA.

• The immune response against PbMSP-9 (NM) was characterized by predominance of IgG1 isotype, followed by IgG2b, IgG2a and IgG3, regardless of the strain of mice and the adjuvant used.

• PbMSP-9 (NM) induced cellular immune response with predominance of IFN\(\gamma\) and variable levels of the regulatory cytokine IL-10, among different immunization groups.

• Mice immunized with PbMSP-9 (NM) were intravenously challenged with \textit{P. berghei} NK65 asexual blood-stage parasites. Complete protection against blood-stage parasite challenge was observed in BALB/c mice immunized with PbMSP-9 (NM) formulated in Alum, in contrast with BALB/c-CFA, C57BL/6-CFA and C57BL/6-Alum groups where no protection was observed.

• BALB/c-Alum group showed the highest level of IFN\(\gamma\) (3548 ± 25 pg/ml) compared with other groups. Thus, recombinant PbMSP-9 (NM) formulated in Alum elicited a Th-1 type immune response that conferred 100% protection in BALB/c mice.

In conclusion, this is the first study reporting the evaluation of the protective efficacy conferred by a member of the MSP-9 family in a homologous system for immunization and challenge. We have shown that PbMSP-9 (NM) recombinant protein is an effective vaccine antigen, with the ability to induce immune response that was able to protect BALB/c mice challenged with the lethal strain \textit{P. berghei} NK65. However, the observed protection was influenced by the type of adjuvant used for the immunization and the genetic background of the host.