INTRODUCTION
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In spite of all the efforts of governments and scientists, malaria continues being the main cause of morbidity and mortality in many undeveloped countries throughout the world. There are at least 300 - 660 million cases of acute infections every year, resulting in approximately one million deaths (Snow et al., 2005). Out of this, around 90% occur in children under 5 years living in African countries (Roll Back Malaria, 2005).

Malaria is caused by an intracellular parasite of the genus *Plasmodium* and is transmitted by the infected female of *Anopheles* mosquito during blood meals. Four species affect human health: *Plasmodium falciparum, P. vivax, P. malariae and P. ovale*. The most severe and life-threatening form of the disease is caused by *Plasmodium falciparum*, which is transmitted mainly in Africa. On the other hand, *Plasmodium vivax* is the most widespread of the four species, producing causing morbidity in Africa, Asia and Latin America (WHO, 2005). Control measures have been traditionally based on the reduction of the transmission by the use of insecticides against the mosquito vector, chemoprophylaxis to prevent the development of severe disease and early treatment of diagnosed cases. However, all these efforts remain only partially successful in controlling the disease.

During the last fifty years, an alternative approach based on the development of an effective malaria vaccine has been pursued. Efforts have been focused on three vaccine types derived from the life cycle stages of the parasite: pre-erythrocytic vaccines, asexual-blood stage vaccines and sexual stage vaccines.

Asexual blood-stage or erythrocytic stage vaccines are intended to block the process of invasion of erythrocytes by merozoites. This type of vaccine does not prevent the infection but is expected to reduce the level of parasitemia and decrease the incidence of severe disease. In this context, parasite antigens located at the merozoite surface have been prioritized as potential vaccine candidates.

A total of ten merozoite surface proteins have been identified until now in *Plasmodium falciparum*, along with homologues of some of them in other species of *Plasmodium*. The most well studied antigens include merozoite surface protein 1 (MSP-1), MSP-2,
MSP-3, and Apical Membrane Antigen 1 (AMA-1). Antibodies against these molecules are able to block the invasion of merozoites. MSP-1, AMA-1, and MSP-3 have been produced as candidate vaccines and have been shown to protect non-human primates from asexual stage parasitemia when administered with Freund's complete adjuvant (Ballou et al., 2004).

In 1986 Stahl et al. (1986) reported the identification of a novel *P. falciparum* antigen of ~101 kDa molecular weight, characterized by the presence of hydrophilic dipeptide and tripeptide repeats of glutamic acid and lysine; for this reason it was named as Acidic Basic Repeat Antigen (ABRA). The major reason to consider ABRA as a promising vaccine candidate was based on the fact that the protein was identified as part of the immune complexes formed in vitro when the released merozoites were in contact with human immune sera (Chulay et al., 1987; Lyon et al., 1986), indicating that ABRA was the target of natural immune response in individuals from endemic areas.

On the other hand, Nwagwu et al. (1992) observed an apparent autoproteolysis of native ABRA that was inhibited by chymostatin and they also showed that the purified native protein possessed chymotrypsin-like activity, suggesting its putative role as a protease involved in the invasion process. With the discovery of ABRA homologues from simian parasite species, the new Merozoite Surface Protein-9 family was defined (Vargas-Serrato et al., 2002). Today after 20 years of research on *P. falciparum* MSP-9 (ABRA), many questions regarding the role of this antigen in the parasite biology and its potential to confer protective immunity are still unsolved. However, a major hurdle in establishing a protein as a vaccine candidate is the unavailability of animal model systems to carry out challenge studies.

Keeping this information in mind, we made an attempt to establish a mouse model of immunization and homologous challenge to assess the vaccine potential of a Merozoite Surface Protein-9. The thesis work presented here was carried out to accomplish the following objectives:
OBJECTIVES

1. To identify and characterize the msp-9 genes from *P. berghei* and *P. yoelii*.

2. To express and purify the full-length recombinant *P. berghei* MSP-9 and its fragments using the *E. coli* expression system.

3. To carry out biochemical characterization of these proteins vis-à-vis protease activity and interaction with the host erythrocyte.

4. To evaluate the immunogenicity (humoral and cellular immune responses) of recombinant PbMSP-9 or its fragments in mice of two different genetic backgrounds.

5. To assess the protective efficacy of the immune response elicited by *P. berghei* MSP-9 recombinant protein against the challenge with the homologous strain *P. berghei* NK65 in two genetically different strains of mice.