TOWARDS BRAZILIAN *PLASMODIUM*-BLOCKING MOSQUITOES

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The Oswaldo Cruz Foundation (FIOCRUZ) is part of the National Health Ministry and a research institute internationally recognized for its basic and applied research in tropical diseases. Research, technological development, production of vaccines, immunobiologicals and pharmaceuticals, quality control, training of human resources and health care service form the basis of its activities. The unit of FIOCRUZ that I belong to is the René Rachou Research Center (CPqRR, Belo Horizonte, MG) that develops strong activities in the field of parasitological research, including studies on schistosomiasis, leishmaniasis, Chagas Disease and Malaria (*www.cpqrr.fiocruz.br*). This unit possesses modern laboratorial infrastructure, equipped for the accomplishment of methodologies in molecular biology, immunology, cell biology/ ultra structure and molecular entomology.

Over the past 10 years, the CPqRR Malaria laboratory, headed by Dr. Antoniana U. Krettli has conducted a number of immunoepidemiological studies in Malaria endemic areas of Brazilian Amazon. These studies have been undertaken to characterize the natural immune response against sporozoite and blood-stage antigens in different situation of transmission. This lab demonstrated a cross-reactive cellular immune response to circumsporozoite (CS) proteins in Malaria-exposed individuals, while the same individuals displayed antigen specificity for the antibody response.

Other research lines include the prospection of phytochemicals for the generation of Malaria medicines, where plants with promising antimalarial effects have been characterized and also the search for novel mosquito vector species in Malaria endemic areas in Brazil.

In Brazil, in 1999, the governmental health agency reported more than 600,000 cases of Malaria, which after hard campaign it is been equilibrated in around 350,000 case a year. The problems related to parasite drug resistance, insecticide resistance by the vectors and the lack of an effective vaccine show the importance to search for alternative means of controlling this deadly disease.

The mosquito is the obligatory vector for Malaria transmission. After the mosquito ingests an infected blood meal, the parasite undergoes a complex developmental cycle, which includes mating of the parasite gametes and the transformation into three different forms: ookinete, oocyst and sporozoites and also the crossing of two different epithelia (midgut and salivary glands) (Ghosh et al., 2000). This complex development within the mosquito offers multiple potential targets to interfere with transmission.

The insertion of foreign genes into the genome of an organism is an important tool not only to study the expression of the gene itself but also for practical uses. Transgenesis is a novel tool with potential use for the control of vector-transmitted diseases (Coates et al., 1998; Catteruccia et al., 2000; Moreira et al., 2000; Kokoza et al., 2000; Grossman et al., 2001). Recently, Ito et al. (2002) and Moreira et al. (2002) demonstrated that a synthetic peptide (SM1) and the bee venom phospholipase A2, respectively, can interfere with *Plasmodium* development in mosquitoes. While these results are encouraging, it is important to consider that the *Plasmodium* genome is known for its plasticity (Gardner et al., 2002) and the possibility of the emergence of resistant parasite strains is a likely possibility. For this reason, it will be important to

develop multiple effector genes that can interfere with parasite development in mosquitoes by independent mechanisms.

We are constructing an hybrid gene using the midgut specific carboxypeptidase promoter (Edwards et al., 1997) linked to an antiparasitic gene, called gomesin, as an alternative parasite blocking effector molecule and also we intend to adapt the *Anopheles stephensi* microinjection technique to Brazilian species of anophelines, e.g. *An. albitarsis* and/or *An. aquasalis*.

While searching for alternative antiparasitic genes we found in the literature the antimicrobial peptide gomesin, isolated from a spider and shown to strongly affect the bacterial growth and the development of fungi and yeast and also the viability of the parasite *Leishmania amazonensis* (Silva et al., 2000). When we tested this peptide against the *P. berghei* on mouse system by injecting intravenously different concentrations of gomesin in infected mice, 45 to 86% less oocysts were detected in mosquitoes that fed on the injected mice, in comparison with the pre-injected group of mosquitoes.

To test the effect of gomesin on the exflagellation of *P. berghei*, different concentrations of the peptide were added to the culture medium and exflagellation events were measured, showing that this peptide greatly reduced the number of exflagellation events.

Based on these preliminary results, we concluded that the gomesin is a promising candidate as an alternative effector gene and its effect *in vivo* needs to be explored, by the generation of anopheline transgenic mosquitoes expressing this molecule with the use of the transposon 3xP3-EGFP piggyBac (Horn and Wimmer, 2000). First we intend to use the *An. stephensi* species because of the facility of generating transformants. As we cannot maintain this species in Brazil, we will perform the first phase transformation experiments in Dr. Jacobs-Lorena's laboratory (USA), where we made this technique routine. Concomitantly, we will work on the transformation of Brazilian anopheline vectors, in our lab. Then, the Brazilian transgenic mosquitoes will be tested in Malaria endemic areas with *Plasmodium vivax* and *P. falciparum* infected blood for their capacity of parasite blockage.

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