

Aspects of *Aedes-Plasmodium* Interaction

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Current Research Interests:

1. Effects of malaria infection on vitellogenesis in *Aedes aegypti*.

The development of transgenic mosquitoes expressing antipathogen effector molecules, and their ability to block or reduce disease transmission under laboratory conditions, has fostered enthusiasm for releasing these insects as a tool to increase the efficiency of integrated insect vector control program. Selection criteria for genes for which promoters could drive sustainable expression of transgenes include those that have a high level of constitutive expression. However, such expression patterns and product accumulation may have a negative physiological effect on larval and pupal stages when effector molecule expression is not needed. Furthermore, infections by different pathogens have been shown to reduce reproductive fitness of infected mosquitoes compared with uninfected ones. Using *Plasmodium gallinaceum* infected *Aedes aegypti* we have analyzed differences in mRNAs profiles that reflect changes in transcriptional activity of genes during infection. We monitored concentration of 10 different transcripts during the first gonotrophic cycle of infected and uninfected mosquitoes by RT-PCR. Our results showed that *P.gallinaceum* infection results in decrease on temporal abundance of vitellogenin (Vg) (60%), vitellogenic carboxypeptidase (VgCx) (30%) and vitellogenic cathepsin B (VgCt) (25%) mRNAs, all of them produced by fat bodies. It is known that these transcripts are regulated by 20-Hydroxyecdysone (Raikhel et al., 2002). On the other hand, no differences were observed on mRNA levels of ovary transcribed genes, as those coding for Vg-receptor (VgRc) and lipophorin-receptor (LpRc). In addition, the experimental protocol was used to compare mRNA abundance of lipophorin (Lp) and transferrin (Tr), major transport proteins and defensin A (Df) synthesized by fat bodies, and ferritin (Fr) that is synthesized by midgut cells. Our results show that during the first gonotrophic cycle, no differences on profile of Lp, Tr, Df and Fr transcripts were detected between control and infected mosquitoes.

Another approach in our research group is the study of infected *Aedes aegypti* hemolymph proteins using proteomic methodology. Proteomics offers an excellent way to examine the host genome in action through qualitative and quantitative evaluations of the mosquito proteins during the host-parasite interaction process. Using the first generation of proteomic tools (2DPAGE – Mass Spectrometry – MS) we are analyzing 100 spots (polypeptides). We are using Image Master 2D

Platinum to make a two dimensional profile off the gels. Our preliminary data show that in the hemolymph of *P. gallinaceum* infected mosquitoes 16 spots have no difference on the expression while 6 spots increase and 6 spots decrease in concentration. We also found that 5 spots are expressed only in non-infected mosquitoes and 12 spots are detected only on infected mosquitoes. The mass spectrometry analyses are ongoing and offer a new way to understand host-parasite interaction.

2. Effector molecules

A number of effector mechanisms have been carried out to interfere with the different developmental stages of malaria parasites in mosquitoes (Nirmala & James, 2003). In an attempt to target sporozoites, we are studying molecules that interacted with the parasites by different mechanisms.

The first group of molecule is comprised of competitor peptides that bind to salivary glands receptors blocking sporozoite invasion. We are using as model for designing these peptides the *Plasmodium ssp* CSP and TRAP, which have been assigned as playing major roles in the sporozoites invasion process.

The second group consists of peptides with parasitocidal effects killing the sporozoites in the hemolymph or salivary glands of the mosquitoes. With this aim we searched for human blood peptides that interact with malaria sporozoites in mosquitoes and in vertebrate host. We tested synthetic peptides based on hemoglobin, fragment (αHb_{33-61}), and Angiotensin II. Our results show that both peptides are highly active against immature and mature *P.gallinaceum* spzs at a concentration of 30 and 60 μM , respectively. More than that, we have designed 6 Angiotensin II analogues (VC 1 to 6) that lose their pharmacological properties in vertebrates but maintain the anti-spzs activity. One of these peptides (VC 5) was tested for its ability to prevent malaria infection in vertebrate host. Fifty or 500 spzs treated *in vitro* with VC 5 (60 μM) were injected in 10 chicks and the infection monitored for 20 days. Nine out of the ten sporozoite challenged chicks were protected, not developing malaria infection.

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