Apical Membrane Antigen 1 (AMA-1): Role in *Plasmodium yoelii* Infectivity

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Introduction

Plasmodium enters the blood stream of a mammalian host via a bite by an infected *Anopheles* mosquito. Translocation to the liver and introduction into a hepatocyte is a critical step for infectivity of the malaria parasite. Entry of the parasite follows two distinct pathways: rupturing of the hepatocyte membrane by migration or by the adhesion, internalization, and formation of a vacuole within the hepatocyte (Silvie et. al., 2004b). Only the latter pathway is necessary for the differentiation and proliferation of the blood-stage pathogen.

My focus will be primarily on the interaction of cell-surface proteins between the hepatocyte and the parasite. There are two well-studied proteins secreted by apical micronemes (i.e. vesicles at the anterior tip of the protozoan which secrete enzymes for parasite entry): the circumsporozoite protein (CSP) and the thrombospodin-related adhesive protein (TRAP; Silvie et. al., 2004b). The exocytosis of CSP and TRAP from micronemes within the parasite is dependent on the transient increase of intracellular calcium. Once the micronemes are excreted, CSP and TRAP localize to the membrane of *Plasmodium*. This process exposes CSP and TRAP to interact with hepatocyte cell-surface proteins, thus allowing the internalization and infection of the parasite by an unknown mechanism. A parasitophorous vacuole (PV) is formed after internalization, which is required for the differentiation of the exoerythrocytic form (EEF; Silvie et. al., 2004b).

CSP has many roles in the life of *Plasmodium* including sporozoite formation in oocysts, gliding locomotion, hepatocyte invasion, as well as inhibition of ribosomes (Menard, 2000). During gliding motility, CSP is shed from the cell surface. Previous studies have used monoclonal antibodies (mAb) against CSP and prevented locomotion of *Plasmodium*, thereby preventing infection (Mota et. al., 2002b). CSP's array of functions present many therapeutic targets. *Plasmodium falciparum* CSP vaccines have had some triumph in human trials, yet effective blood titer of antibody were not sustained (Gantt et. al., 2000). Yet, success has not been observed with TRAP because its exposure is limited to the intimate contact with unknown hepatocyte cell-surface proteins. CSP, on the other hand, is evenly distributed on *Plasmodium,* allowing for more binding sites on hepatocytes for antibody neutralization.

Much success has been achieved with the study of TRAP and CSP. These accomplishments give basis for further study of other parasite-surface proteins. Apical Membrane Antigen 1 (AMA-1) is an understudied protein also found within micronemes and is released onto the cell-surface with the advent of intracellular calcium. Silvie and colleagues (2004a) have inhibited HepG2 cell *Plasmodium falciparum* infectivity with a mAb against AMA-1. I propose to perform a similar experiment using a primary hepatocyte cell culture from mice with *Plasmodium yoelii*. Further understanding of AMA-1 role in parasite entry will allow us to develop a potential synergistic vaccine with a CSP inoculum in a murine model, which cannot be immediately done in HepG2 cell cultures (Gantt et. al., 2000).

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Aims

Based on evidence seen in HepG2 cells, I will test the efficacy of anti-AMA-1 mAb in mice to neutralize the infectivity of *Plasmodium yoelii* in primary hepatocyte cultures from wild-type mice. Previously, HepG2 cells were protected against *Plasmodium* infectivity with increasing concentrations of anti-AMA-1 mAb (Silvie et. al., 2004a).

Secondly, I will test the infectivity of *P. yoelli* on primary hepatocytes from mice preincubated with anti-AMA-1 mAb and treated 3 hours post sporozoite introduction. In the past, HepG2 cells were found susceptible to *Plasmodium* infection after this procedure (Silvie et. al., 2004a). . This study will further verify the necessity of the AMA-1 protein for sporozoite entry into hepatocytes and subsequent infection.

Experimental Procedure

Firstly, I hypothesize that the anti-AMA-1 mAb will prevent *Plasmodium yoelii* entry and infection of wild-type mouse hepatocytes as noted in HepG2 cells (Silvie et. al., 2004). The anti-AMA-1 mAb for *P. yoelii* will be obtained as described in Silvie and colleagues (2004a). Note, the ectodomain of AMA-1 is conserved across many *Plasmodium* species including parasites that infect rats. Primary hepatocyte cultures from wild-type mice will be treated with anti-AMA-1 mAb and inculated with *P. yoelii* sporozoites in the presence of rhodamine-labeled dextran (Silvie et. al., 2003c). Dextran-positve cells will indicate the migration of sporozoites without parasitophorous vacuole (PV) formation. Dextran-negative cells will indicate PV formation without disruption of the hepatocyte membrane.

I believe that AMA-1 is necessary for *Plasmodium* invasion and proliferation in the hepatocyte, thus treated cells should be Dextran-positive (i.e. noting the migration of parasites thru hepatocytes without PV formation due to mAb competitive inhibition). One pitfall may be the presence of calcium in this procedure. Calcium has not been added to induce exposure of the AMA-1 protein from the microneme. A calcium ionophore may be necessary to trigger the apical exocytosis upon invasion (Mota et. al, 2002a). A green phycoerythrin labeled monoclonal antibody will be used to stain for the presence of the PV, or circumsporozoite proteins (CSP) as described in Silvie and colleagues (2003c) thus noting infectious entry. A culture of *P. yoelii* infected cells not treated with the anti-AMA-1 mAb will serve as the control for this experiment. The control cells should be both Dextran-negative and Dextran-positive because of the two invasion pathways observed by *Plasmodium*: migration and infectious entry.

Secondly, I will further verify the role of AMA-1 in *Plasmodium* infectivity by preincubating a primary wild-type mouse culture with anti-AMA-1 mAb and *P. yoelii*. Microscopy as well as immunofluorescence decribed above will be used to assess the percentage of exoerythrocytic forms (i.e. blood stage pathogens) within hepatocyte tissue. This experiment is significant because it will assess the level of toxicity induced by the antibody treatment on *P. yoelli*. I hypothesize no significant induced toxicity of *P. yoelii*. This will serve as the control for the following experiment. Primary culture, wild-type mouse, and hepatocytes will be inoculated with *P. yoeli,i* which will be incubated for 3 hours. After this time period, anti-AMA-1 mAb will be used to treat the infected hepatocytes. The percent of EEFs will be monitored for a 48 hour period.

I hypothesize a significant increase of EEFs in comparison to the control. If the result is positive, this experiment will confirm the necessity of the AMA-1 protein for invasion and formation of a PV for *Plasmodium* proliferation. If negative, the AMA-1 protein may be operating in an undetermined mechanism. This mechanism may be similar to the TRAP protein, in that, exposure of AMA-1 from the apical complex occurs only after the influx of calcium and close proximity to the hepatocyte. Thus, the antibody treatment may not present a satisfactory way to bind AMA-1 because of the discrete affinity of AMA-1 to unknown cell-surface proteins on the hepatocyte. This proposed model contrasts the uniform arrangement of CSP around *Plasmodium*.

Conclusion

The above experiments have been used previously to elucidate the significance of parasitophorous vacuole formation for infectivity of *Plasmodium* in Silvie and colleagues (2003c). With the conclusion of the above research, we will have further characterized the function of an additional microneme protein in context with sporozoite infectivity. In addition to TRAP and CSP, AMA-1 may serve as a putative receptor for sporozoite invasion and be used to construct a vaccine similar to the human CSP vaccine. A combined CSP/AMA-1 immunization may prove to have synergistic effects on Plasmodium protection (Gantt et. al., 2000).

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References

Gantt, S., Persson, C., Rose, K., Birkett, A. J., Abagayan, R., and Nussenzweig, V. (2000). Antibodies against thrombospondin-related anonymous protein do not inhibit *Plasmodium* sporozoite infectivity in vitro. Infect. Immun. 68, 3667–3673.

Menard, R. (2000). The journey of the malaria sporozoite through its hosts: two parasite proteins lead the way. Microbes Infec. 2, 633-642.

Mota, M.M., Hafala, J.C.R., and Rodriguez, A. (2002a). Migration through host cells activates *Plasmodium* sporozoites for infection. Nat. Med. 8, 1318-1322.

Mota, M. M., and Rodriguez, A. (2002b). Invasion of mammalian host cells by *Plasmodium* sporozoites. BioEssays 24, 149-156.

Silvie, O., Franetich, J. F., Charrin, S., Mueller, M. S., Siau, A., Bodescot, M., Rubinstein, E., Hannoun, L., Charoenvit, Y., Kocken, C. H., Thomas, A. W., Van Gemert, G. J., Sauerwein, R. W., Blackman, M. J., Anders, R. F., Pluschke, G., and Mazier, D. (2004a). A role for apical membrane antigen 1 during invasion of hepatocytes by *Plasmodium falciparum* sporozoites. J. Biol. Chem. 279, 9490-9496.

Silvie, O., Franetich, J.F., Renia, L., and Mazier, D. (2004b). Malaria sporozoite: migration for a living. Trends Molec. Med. 10, 97-100.

Silvie, O., Rubinstein, E., Franetich, J.F., Prenant, M., Belnoue, E., Renia, L., Hannoun, L., Eling, W., Levy, S., Boucheix, C., Mazier, D. (2003c). Hepatocyte CD81 is required for Plasmodium falciparum and Plasmodium yoelii sporozoite infectivity. Nat. Med. 9,93-96.