Cap380 as a marker for *Plasmodium falciparum* oocyst development *in vivo* and *in vitro*

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ABSTRACT

Despite the importance of the *Plasmodium berghei* oocyst capsule protein (PbCap380), very little is known about the orthologous *Plasmodium falciparum* (*Pf*) capsule protein (PfCap380).

Here, we present *in vitro* methodology for production of early *Pf* oocysts and a reagent to study oocyst development, (i.e., an antibody against an oocyst surface protein, Cap380). As oocysts develop, they secrete several proteins, and these secreted proteins along with mosquito-derived factors form a non-bilayer structure surrounding the oocysts called the capsule. One of the oocyst-derived capsular proteins was described in *P. berghei*, PbCap380, and was found to be essential for sporozoite development [1]. Cap380 is the only stage-specific oocyst marker known and antibodies against Cap380 would improve *in vitro* sporozoite culturing systems by allowing for oocyst-stage detection and quantification and determination of transformation rates from ookinetes to early oocysts. Prior to these studies, an antibody against PbCap380 was developed, but due to low homology between *Pb* and *Pf* of the immunogenic region that was used to raise the anti-PbCap380 antibody, anti-PbCap380 does cross react with the *Pf* oocyst capsule [1].

Here, we demonstrate the utility of the anti-PfCap380 antibody to study the development of *P. falciparum* oocysts *in vivo* and *in vitro*. The aims of this study include detecting early oocysts and following the developmental progression of oocysts using PfCap380 as a marker. This study should enable the standardization of *in vitro* culture systems that produce the mosquito equivalent stages, oocysts and functional sporozoites, for scalable production of whole-SPZ based vaccines, including genetically attenuated parasites (GAP) [2].

Flow diagram of *in vitro* oocyst culture

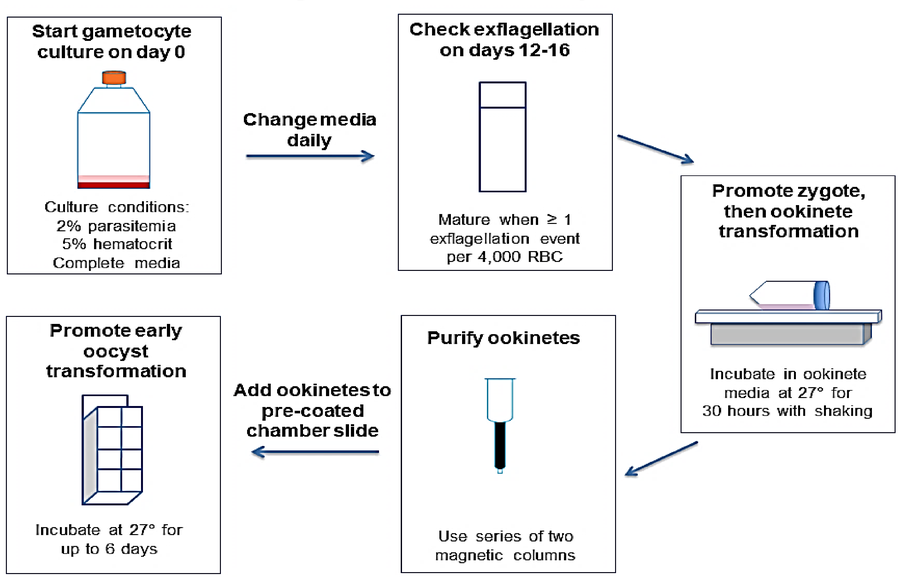

Figure 2. Gametocyte culture was seeded from an asexual culture in complete media (RPMI with 10% A+ human serum). Once the culture was matured, flasks were combined, pelleted, and resuspended in of ookinete media. Zygotes formed after five hours and ookinetes transformed after of 30 hours of incubation 27°C and shaking at 50 RPM. Ookinetes were purified using magnetic columns, resuspended in oocyst media, and plated into 8-well-chamber slides that were pre-coated with basal lamina components. Oocysts were incubated at 27°C for up to 6 days in oocyst media.

Expression of PfCap380 in early *in vitro* produced oocysts

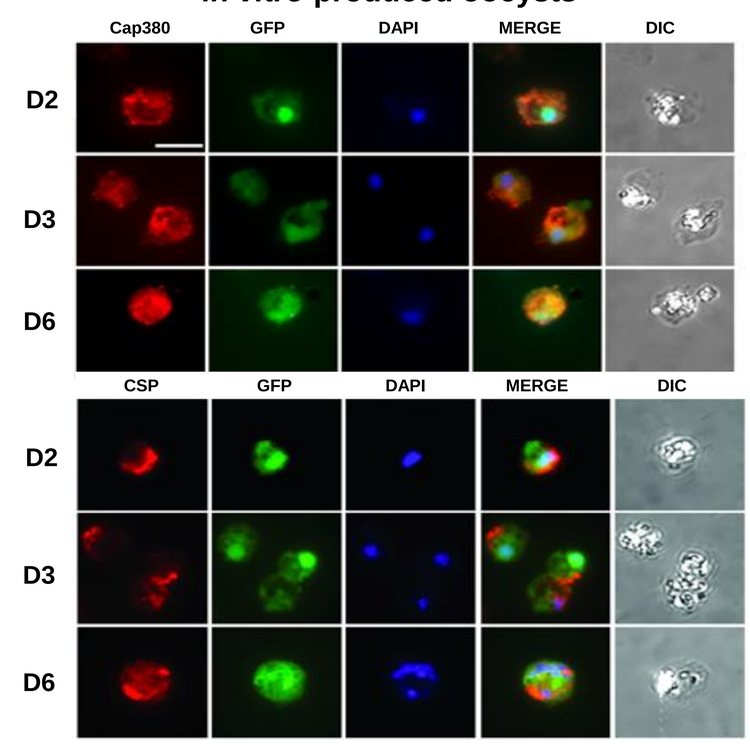

Figure 3. IFAs were performed on *in vitro* oocysts as indicated time points, (D = Day). Oocysts were labeled with primary antibody (red) against PfCap380 or PfCSP, GFP in green and DAPI nuclear staining in blue. Scale bars = 5 µm.

RESULTS and METHODS

The anti-PfCap380 antibody allowed detection of early midgut oocysts starting at day 2 after gametocyte infection, while circumsporozoite protein (CSP) was strongly observed on day 6. CSP was found to be localized just under PfCap380. For the *in vitro* culture, we observed a moderate transformation of gametocytes to ookinetes (24%) and a high transformation of ookinetes to early oocysts (85%). After screening several basal lamina components, a combination of collagen IV, laminin, and entactin supported greater binding capacity of ookinetes and transformation into early oocysts. Finally, PfCap380 expression was observed only in oocysts and not in other stages (i.e., gametocytes and ookinetes).

To study *Pf* oocyst development using both *in vivo* (mosquito-derived) and *in vitro* (culture-derived) growth conditions, a polyclonal antibody was raised against PfCap380. For studies on *in vivo* oocysts, mature *Pf* gametocytes were fed to *Anopheles stephensi* mosquitoes. For studies on *in vitro* parasites, *Pf* gametocytes were induced and then transformed into ookinetes. Ookinetes were purified and then tested for binding affinity to basal lamina components and transformation into early oocysts. Early oocysts were grown on the reconstituted basal lamia coated wells with novel oocyst media. To monitor *in vivo* oocyst development, indirect immunofluorescence assays (IFAs) were performed using anti-PfCap380 antibody with *Pf*-infected mosquito midguts. IFAs were also performed on culture-derived oocysts to follow *in vitro* oocyst development.

Expression of PfCap380 and CSP on midgut oocysts

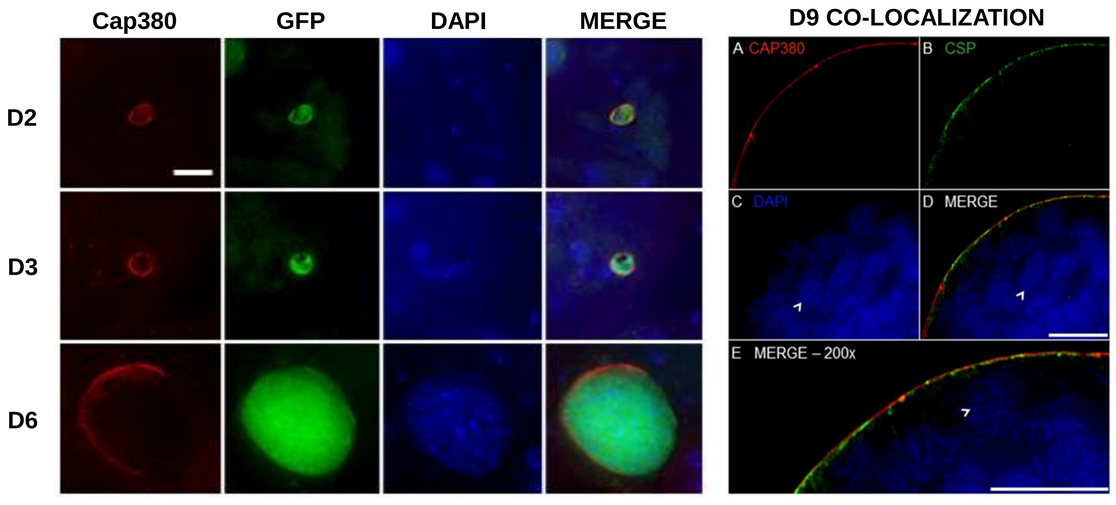

Figure 1. IFAs were performed on GFP-Luc infected midguts at the indicated time points (D = Day). A. Midguts were labeled with primary antibody against PfCap380 (red), and show expression of GFP in green and nuclear staining with DAPI in blue. For co-localization studies, day 9 infected midguts were labeled with primary antibodies against PfCap380 in red (A) and CSP in green (B) and with DAPI in blue to stain nuclei of sporozoites (C). The merged image of the three channels shows the stained capsule with sporozoite nuclei inside (D). Scale bars = 10 µm.

Gametocytes and ookinetes do not express PfCap380

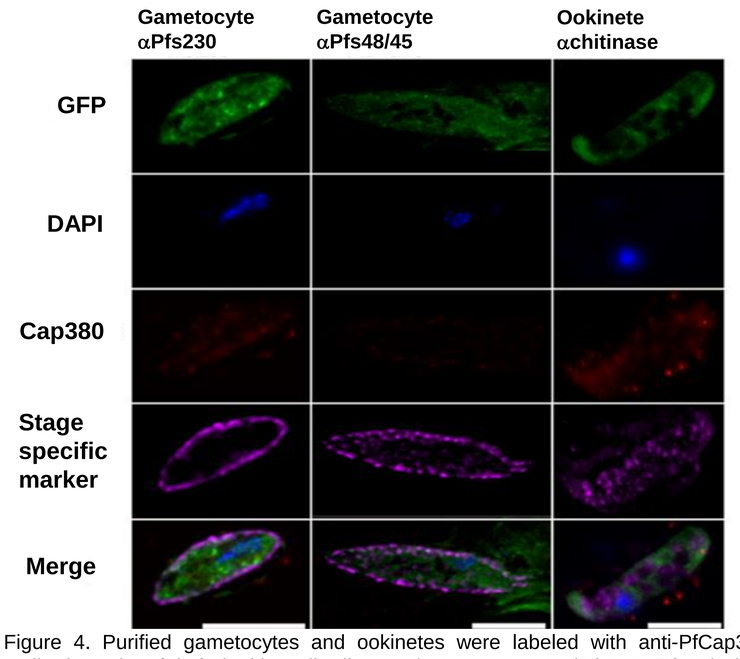

Figure 4. Purified gametocytes and ookinetes were labeled with anti-PfCap380 antibody and co-labeled with antibodies against gametocyte (P*f*s230, P*f*s48/45) or ookinete (chitinase) markers. Gametocytes and ookinetes express GFP in green and nuclei stain with DAPI in blue. PfCap380 is shown in red; Pfs230, Pfs48/45, and chitinase are in purple. Gametocytes do not express PfCap380 but express Pfs230 and P*f*s48/45. Ookinetes show a very faint anti-PfCap380 signal and strong cytosolic expression of chitinase. Scale bars are 10 mm in A; 7.5 mm in B and C.

CONCLUSIONS

- Mosquito-based stages of *Plasmodium falciparum* can be produced using *in vitro* culturing systems
- PfCap380 is expressed on the oocyst capsule of *in vivo* and *in vitro* oocysts
- Development of *in vivo* and *in vitro* oocysts can be monitored using the anti-PfCap380 antibody
- PfCap380 is a useful marker to follow the development and maturation of *in vivo* and *in vitro* produced oocysts as early as day 2 after mosquito infection.
- Further *in vitro* studies focused on oocyst and sporozoite maturation will support the manufacturing of sporozoites for malaria vaccines, including GAP vaccines.

REFERENCES

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2. Kublin JG, et al. (2017). "Complete attenuation of genetically engineered Plasmodium falciparum sporozoites in human subjects." Science Translational Medicine **9**: 1-11.