Anti-CelTOS Transmission Blocking Activity *in vivo* and *in vitro* against *P. falciparum* by Epitope-specific Monoclonal Antibodies

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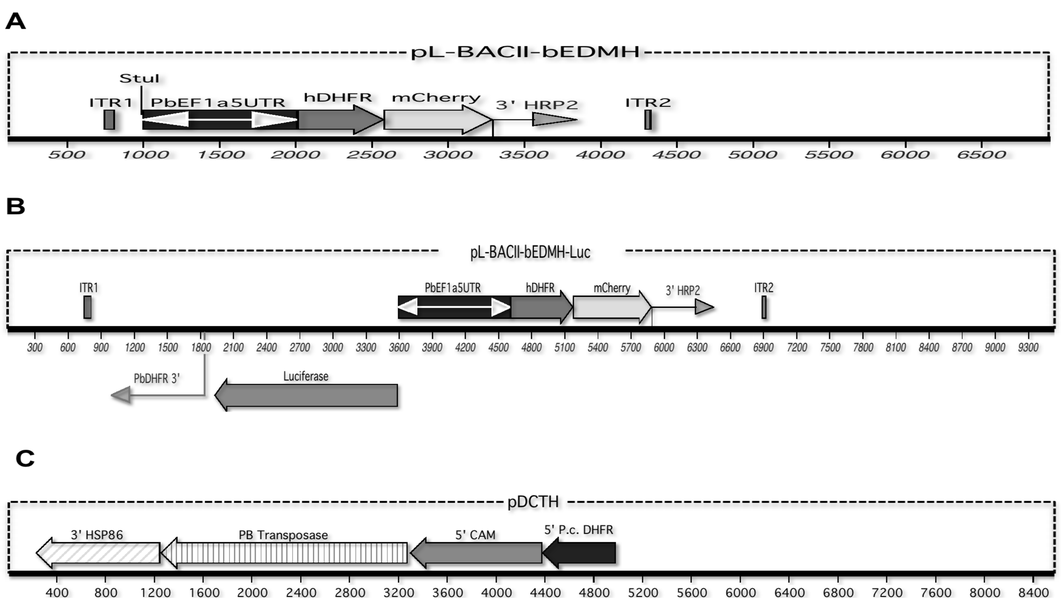
Introduction

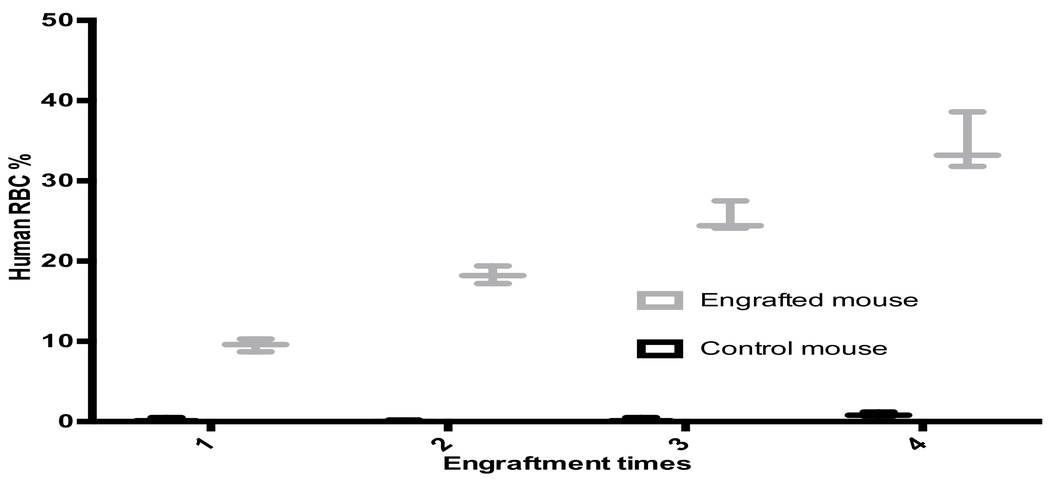
Plasmodium falciparum is responsible for a majority of the morbidity and mortality associated with malaria. Continued success in the global malaria elimination and eradication campaign will require development effective vaccines to prevent malaria transmission[1-3]. The cell-traversal protein for ookinetes and sporozoites (CelTOS) has emerged as a leading transmission blocking vaccine (TBV) candidate[4-5]. We investigated the ability of epitope-specific CelTOS monoclonal antibodies (mAbs) to block transmission of *P. falciparum* sexual blood stages to mosquito stage oocyst using both *in vitro* with a standard membrane-feeding assays (SMFA) and *in vivo* with a *P. falciparum*-humanized mouse model.

Methods and Results

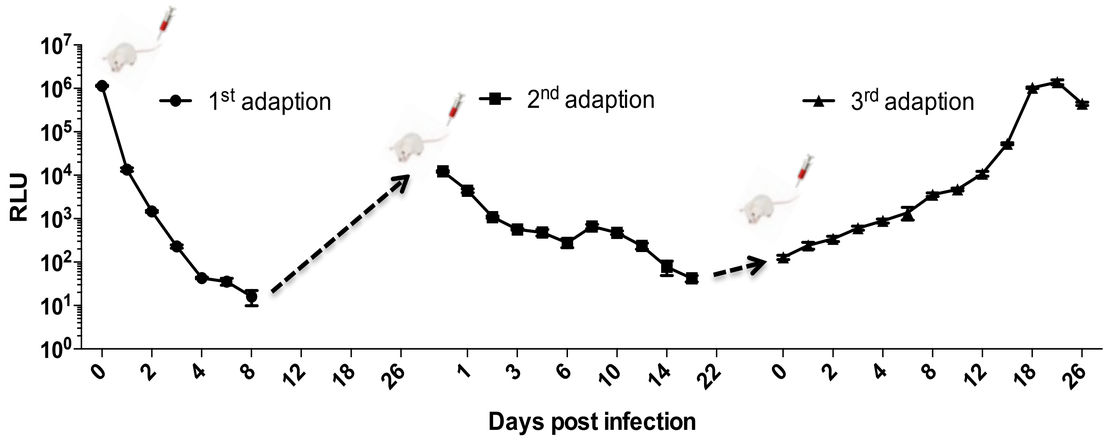
pL-BACII-bEDMH-Luc vector construction and transposase-expressing helper plasmid pDCTH. Luciferase and PbDHFR3' UTR gene was amplified from existing expression cassette and inserted it into a mCherry-hDHFR piggyback vector pL-BacII-bEDMH to obtain pL-BACII bEDMH-Luc plasmid vector. A. Backbone plasmid pL-BACII-bEDMH. B. Luciferase-expressing *piggyBac* vector pL-bEDMH-Luc driven by *P. berghei* EF1-α promoter with hDHFR drug selection marker. C. Transposes-expressing helper plasmid pDCTH with 5'-*P.chabaudi* DHFR-TS and 5'-*P.falciparum* calmodulin (PfCAM) dual promoters.



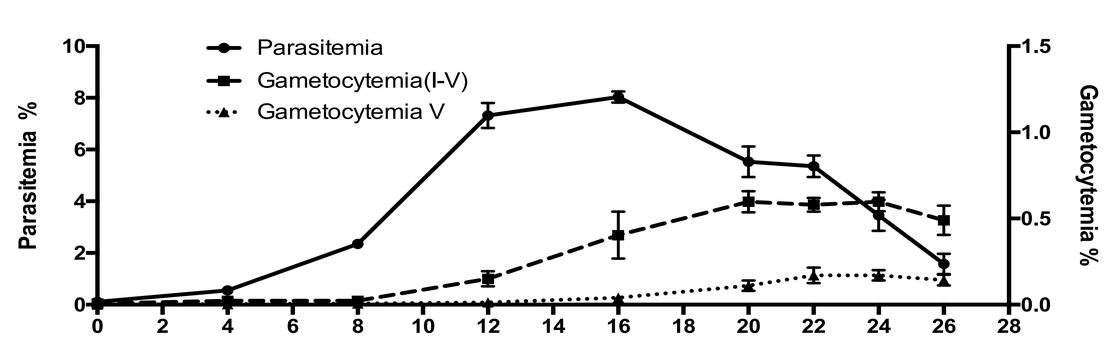
huRBC engrafted mice blood FC analysis by single staining with anti-human CD235a. The error bars represent standard deviation from the mean of 8 measurements of human RBC percentages in mouse peripheral blood by anti-human CD235a-APC flow cytometry analysis.



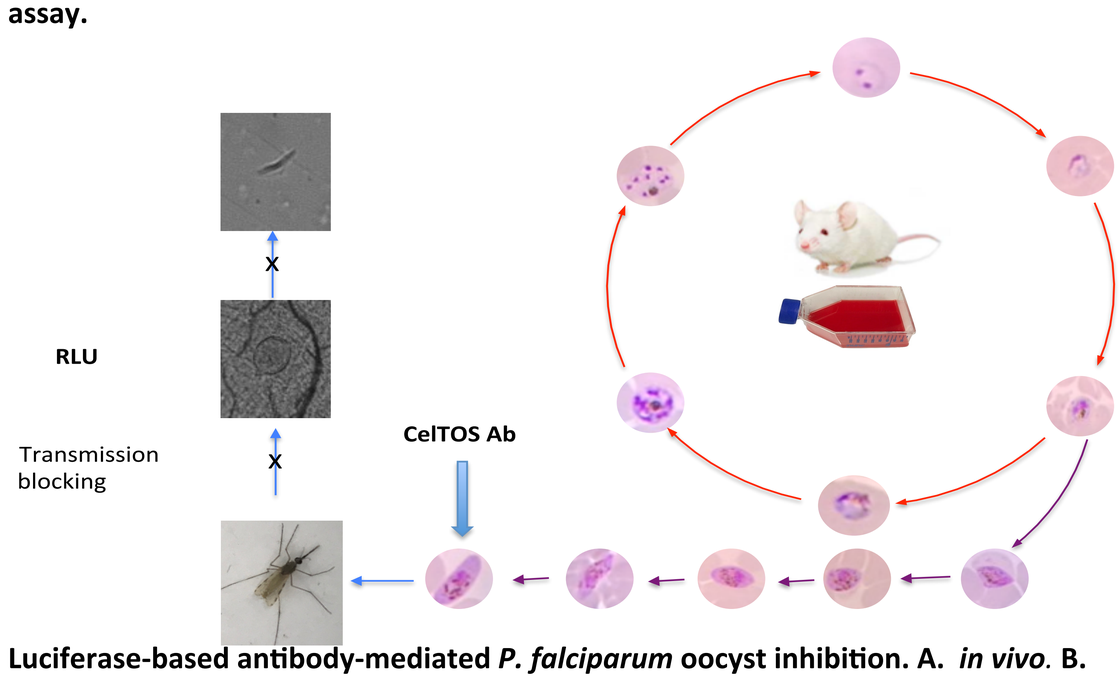
PfKF7G4 parasites were adapted in huRBC-engrafted NSG mice. Independent series of sequential blood to blood *in vivo/vitro* passages were carried out. huRBC-engrafted NSG (huRBC-NSG) mice with peripheral huRBC levels greater than 25% were randomized for adaption experiment. Three mice per group were assigned for each adaption selection. 3rd adaption RLU mean±SD=1399437±173885 on day 22 post infection when the mean of mouse blood thin parasitemia is 10.58%±0.59(n=3).



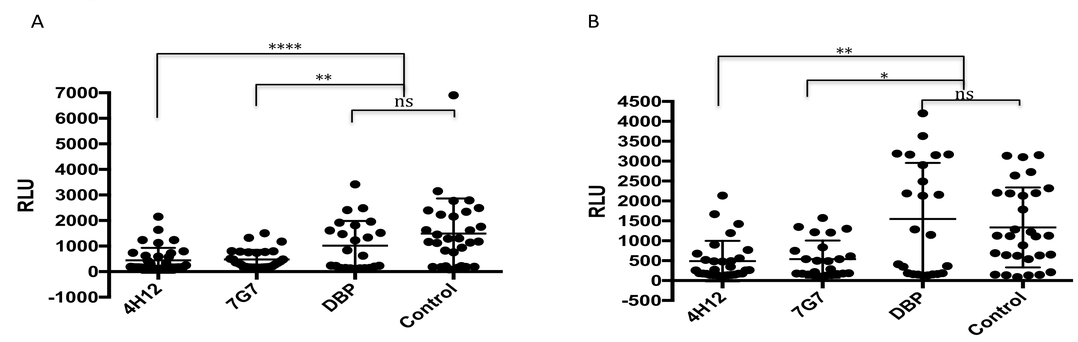
P. falciparum gametocyte culture *in vivo* with mouse adapted PfKF7G4 parasite.



The strategy for oocyst inhibition in *vivo/in* vitro based on PfKF7G4 luciferase assay.



Luciferase-based antibody-mediated *P. falciparum* oocyst inhibition. A. *in vivo*. B. *in vitro*



Summary

Standard in vitro culture methods were used to produce mature gametocytes for the SMFA. The in vivo studies relied on an NF54 line carrying a luciferase-expressing cassette integrated in the genome. NSG mice treated with clodronate lipsome supported high-level engraftment of huRBC and can be infected by this P. falciparum luciferase reporter line, including development of mature gametocytes infectious for mosquitoes and leading to salivary gland sporozoites. This in vivo laboratory model permitted a highly sensitive transmission blocking assay to reliably quantify early oocyst development on day 22 post infection when stage V gametocytemia reached 0.07-0.18%. Mice were randomly divided into 4 groups and 60 min. before direct mosquito feed, each mouse in the antibody-treatment group received 16 mg/kg mAb in 200µl RPMI by iv injection and the blank control group received of equal volume RPMI. For the in vitro SMFA, 400 µg/ml of CelTOS mAb was added to gametocyte culture 60 min. prior to the mosquito feed. The presence of CelTOS mAb significantly inhibited oocyst development in mosquitoes in both in vivo and in vitro assays. Importantly, the experimental results with an innovative in vivo humanized mouse model confirmed that circulating anti-CelTOS antibody effectively inhibits P. falciparum ookinete development to oocyst in mosquitoes. These results support the development of CelTOS as a transmission blocking vaccine.

Study approved by USF Research Integrity and Compliance IACUC

References

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