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Research paper

Impact of exposure to mosquito transmission-blocking antibodies on *Plasmodium falciparum* population genetic structure

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ABSTRACT

Progress in malaria control has led to a significant reduction of the malaria burden. Interventions that interrupt transmission are now needed to achieve the elimination goal. Transmission-blocking vaccines (TBV) that aim to prevent mosquito infections represent promising tools and several vaccine candidates targeting different stages of the parasite's lifecycle are currently under development. A mosquito-midgut antigen, the anopheline alanyl aminopeptidase (AnAPN1) is one of the lead TBV candidates; antibodies against AnAPN1 prevent ookinete invasion. In this study, we explored the transmission dynamics of *Plasmodium falciparum* in mosquitoes fed with anti-AnAPN1 monoclonal antibodies (mAbs) vs. untreated controls, and investigated whether the parasite genetic content affects or is affected by antibody treatment. Exposure to anti-AnAPN1 mAbs was efficient at blocking parasite transmission and the effect was dose-dependent. Genetic analysis revealed a significant sib-mating within *P. falciparum* infra-populations infecting one host, as measured by the strong correlation between Wright's F_{IS} and multiplicity of infection. Treatments also resulted in significant decrease in F_{IS} as a by-product of drop in infra-population genetic diversity and concomitant increase of apparent panmictic genotyping proportions. Genetic differentiation analyses indicated that mosquitoes fed on a same donor randomly sampled blood-circulating gametocytes. We did not detect trace of selection, as the genetic differentiation between different donors did not decrease with increasing mAb concentration and was not significant between treatments for each gametocyte donor. Thus, there is apparently no specific genotype associated with the loss of diversity under mAb treatment. Finally, the anti-AnAPN1 mAbs were effective at reducing mosquito infection and a vaccine aiming at eliciting anti-AnAPN1 mAbs has a strong potential to decrease the burden of malaria in transmission-blocking interventions without any apparent selective pressure on the parasite population.

1. Introduction

Plasmodium falciparum remains an important pathogen of humans, responsible for the majority of malaria cases and deaths in sub-Saharan Africa (WHO, 2015). The Malaria Control Strategy currently recommended by the WHO (WHO, 2015) relies on the use of artemisinin-based combination therapies (ACTs), intermittent preventive treatment during pregnancy (IPTp) and universal distribution of Long Lasting Insecticidal Nets (LLINs). However, widespread drug (Ariey et al., 2014; Dondorp et al., 2009) and insecticide (Briët et al., 2013; Ranson et al., 2009) resistances have hampered the efforts of malaria control programs and new interventions are needed. In the current perspective of malaria elimination, transmission-blocking vaccines (TBVs) that target the parasite's sexual stages in the mosquitoes, represent promising approaches (Nunes et al., 2014; Sauerwein and Bousema, 2015; Smith et al., 2011). Indeed, blocking *Plasmodium* development within the mosquito would interrupt malaria transmission.

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Several TBV candidates that target the mosquito stages of *Plasmodium* parasites are currently in pre- and clinical development (Sauerwein and Bousema, 2015). The mosquito-based TBV alanyl aminopeptidase N (AnAPN1) works in a similar fashion but targets a mosquito receptor for the parasite (Armistead et al., 2014; Atkinson et al., 2015). AnAPN1 is a glycoprotein expressed at the apical surface of the mosquito midgut epithelium with a role in ookinete invasion and is conserved across divergent anopheline species (Atkinson et al., 2015; Mathias et al., 2013). Antibodies against AnAPN1 were shown to block parasite transmission of *P. berghei*, *P. falciparum* and *P. vivax* in different Old World species of *Anopheles* mosquitoes, with parasite species-specific differences in the antibody efficacy (Armistead et al., 2014; Dinglasan et al., 2007; Mathias et al., 2013). The AnAPN1 structure was recently solved and used to map the relevant transmission-blocking epitopes and predict antibody functional activity (Atkinson et al., 2015). Although it has been observed that complete blockade of *P. falciparum* development can be achieved with anti-AnAPN1 antibodies, the mechanism underlying the transmission-blocking activity remains elusive.

P. falciparum has a unique and complex cycle involving multiple cell divisions both in the mosquito and human hosts. Gametocytes are the sexual stages of the parasites that circulate in the peripheral blood of the vertebrate hosts and importantly, are the only parasitic stage that can infect mosquito vectors. Gametocytes are taken up with the mosquito blood meal and transform into gametes. Fertilization between male and female gametes occurs in the midgut lumen, resulting in a diploid zygote. The zygote evolves into a motile ookinete that traverses the midgut epithelium, reaches the basal lamina, and develops into an oocyst that undergoes nuclear reduction (meiosis). Intense multiplications within the oocyst lead to a large number of haploid sporozoites that are released into the hemolymph when the mature oocyst ruptures. Sporozoites invade the mosquito salivary glands and can be transmitted to a new vertebrate host during the next blood meal.

Oocysts represent a convenient stage for genetic studies: they can be individually dissected from the midgut and they represent a diploid stage from which the parental genotypes can be inferred (Annan et al., 2007; Mzilahowa et al., 2007). In malaria endemic areas, human hosts harbor multiple genotypes of *P. falciparum* and recombination or "outcrossing" during sexual reproduction can occur between genetically-distinct gametes in the mosquito (Annan et al., 2007; Morlais et al., 2015; Nkhoma et al., 2012; Paul et al., 1995). Several studies have reported high levels of inbreeding in oocyst populations from field-collected or laboratory-infected mosquitoes, indicating that sib mating (mating between related gametes) is common (Anderson et al., 2000; Annan et al., 2007; Morlais et al., 2015).

Host and parasite genetic heterogeneities in natural conditions might challenge the predicted efficacy of transmission-blocking interventions (TBIs). Efficacy of the RTS,S/AS01 vaccine was already shown allele-specific and the protective effect depends on the parasite genotype at the target locus, the circumsporozoite protein (Neafsey et al., 2015). In malaria areas with high transmission, mixed-strain infections are common and competition between parasite strains influences the transmission of drug-resistant parasites in the human host as well as the infection success in the mosquito vector (Bushman et al., 2016; Morlais et al., 2015). Vaccines with incomplete protection could also impose a selective pressure and favor the spread of escape/resistant genotypes (Gandon et al., 2001; Read et al., 2015).

In this study, we hypothesized that the administration of anti-APN1 mAbs at suboptimal blocking concentrations would favor particular genotypes of *P. falciparum*. We tested this hypothesis in Cameroon, a malaria endemic area with high *P. falciparum* genetic diversity. We genotyped individual *P. falciparum* oocysts isolated from mosquitoes challenged with anti-AnAPN1 mAbs and from control (untreated) mosquitoes at seven microsatellite loci and compared the parasite genetic structure in these two oocyst groups.

2. Materials and methods

2.1. Ethical statement

The asymptomatic gametocyte carriers were enrolled as volunteers after their parents or legal guardians had provided informed consent. All procedures used in this study were approved by the Cameroonian national ethical committee (statements 2013/02/031/L/CNERSH/SP, 2014/04/440/CE/CNERSH/SP and 2015/04/583/CE/CNERSH/SP).

2.2. Recruitment of *P. falciparum* gametocytes donors

Gametocyte carriers were identified among asymptomatic children aged between 5 and 11 years old who were attending primary schools in the Mfou district; a small town located 30 km South-East from Yaoundé, Cameroon. Blood samples were collected by finger prick from each volunteer and thick blood smears were stained with a 10% Giemsa solution and examined by light microscopy (×100 magnification) for the detection of asexual and sexual parasite stages. Gametocyte density was estimated by counting parasite number against 1000 white blood cells (WBCs), assuming the standard number of 8000 WBCs per μl of blood. Children with asexual parasitaemia exceeding 50 parasites/μl were treated with Dihydroartemisine –piperaquine (Malacur®) according to national guidelines.

2.3. Experimental infections of mosquitoes

We performed direct membrane-feeding assays (DMFAs) using five independent, naturally-infected gametocyte donors. Our local mosquito colony of *An. coluzzii* Ngousso was used for infection experiments. For each volunteer, gametocyte-infected blood was collected by venipuncture into heparinized vacutainer tubes. The blood was centrifuged at 800 rcf at 37 °C for 5 min and the donor serum removed. Transmission-blocking assays were performed using the 4H5B7 mAbs that were previously shown to block *P. falciparum* development in *Anopheles* mosquitoes (Atkinson et al., 2015). 4H5B7 mAbs were tested at final concentrations ranging from 0.5 to 50 μg/ml. Dilutions of 4H5B7 were prepared in AB human serum from a non-immune donor and AB serum alone was used as control. For each assay, antibody preparations were mixed at a 1:1 ratio with the packed red blood cell pellet (50% hematocrit) from a single gametocyte-infected blood donor. Mixtures were delivered into water-jacketed membrane feeders maintained at 37 °C. Mosquitoes were allowed to feed for 30 min; unfed or partially fed females after this time were removed from the study. Fully fed mosquitoes were maintained in the insectary under standard conditions (27 ± 2 °C, 85 ± 5% RH, 12 h light/dark) and fed daily with a 6% sucrose until dissections.

2.4. Mosquito dissection and oocyst DNA extraction

At day seven post-infection, we used a batch of female mosquitoes for each treatment (4H5B7-0.5, 4H5 B7-5, 4H5 B7-50 and AB) to evaluate the efficiency of the mAb concentrations at reducing parasite transmission. Mosquito midguts were dissected, stained with a 0.4% mercurochrome solution and examined under a Leica (Deerfield, IL) light microscope (×20) for oocyst counts. The infection prevalence was computed as the percentage of mosquitoes with at least one oocyst per midgut and the infection intensity as the arithmetic mean of oocyst number in the feeding experiment.

The remaining mosquitoes were used for oocyst genotyping. Midguts were dissected at day 9 post-infection in a PBS/0.1% mercurochrome solution, transferred individually in 1.5 ml microtubes containing 100 μl absolute ethyl alcohol and preserved at −20 °C (Annan et al., 2007). Oocysts were individually dissected from each gut after gradual rehydration, consisting in 30 min in a 70% alcohol bath, 30 min in a 30% alcohol bath and then 30 min in distilled water.