AWARD NUMBER: W81XWH-15-1-0401

TITLE: Malaria Prevention by a New Technology: Vectored Delivery of Antibody Genes

PRINCIPAL INVESTIGATOR: Gary Ketner, Ph.D.

CONTRACTING ORGANIZATION: Johns Hopkins University
Baltimore, MD 21218

REPORT DATE: **October 2018**

TYPE OF REPORT: **Annual**

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2018	Annual	8 Sep 2017 - 7 Sep 2018

4. TITLE AND SUBTITLE

Malaria Prevention by a New Technology: Vectored Delivery of Antibody Genes

5a. CONTRACT NUMBER

5b. GRANT NUMBE
W81XWH-15-1-0401

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Gary Ketner (PI)

E-Mail:gketner1@johnshopkins.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Johns Hopkins University
34th and Charles Streets
Baltimore MD 21218

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Malaria has proven refractory to conventional immunization approaches. This project explores a novel route to induction of anti-malaria immunity: adeno associated virus (AAV) vectored transfer of genes encoding known protective monoclonal antibodies (MAbs) to whole animals. Using a specific technology originally applied to expression of HIV antibodies, we demonstrated that mice can be protected from *Plasmodium falciparum* infection by antibodies against circumsporozoite protein, an antigen found on the surface of the form of the parasite injected by mosquitoes. The current project has two specific aims: 1. Identification of optimal MAbs by construction of additional vectors and assessments of protective efficacy in mice, and 2. Tests of protective efficacy of these MAbs, delivered by AAV vectors, in the non-human primate(NHP) *Aotus nancymaa* challenge model of *P. falciparum* infection. In this period, characterization of five MAb vectors in mice has been nearly completed. NHP trials have been hampered by technical difficulties in reproducing the published challenge protocols. Extensive efforts to establish the challenge model with the published parasite strain have been repeatedly unsuccessful. An account of these efforts is included in the report text. An alternative to the published parasite strain has been obtained, and experiments to test the infectivity of sporozoites of this strain for *Aotus* is underway.

15. SUBJECT TERMS
Malaria, monoclonal antibody, immunization, vaccine, gene transfer, adeno associated virus, AAV, *Plasmodium falciparum,* sporozoite murine challenge model, non-human primate challenge model, *Aotus*

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	10	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

1. Introduction. Malaria is caused by parasites of the genus *Plasmodium* and is responsible for about 500,000 deaths per year, mostly in sub-Saharan Africa and mostly induced by infection with *P. falciparum*. In addition to the burden it imposes on residents of endemic areas, malaria poses a significant threat to US service personnel serving in Africa and other malaria-endemic areas. An effective vaccine would be of enormous value in relieving the toll exacted by malaria. However, extensive efforts to develop malaria vaccines using conventional approaches have been largely unsuccessful and no satisfactory malaria vaccine exists. The long-term objective of this project is to assess the promise of a novel immunization technology termed vectored immunoprophylaxis (VIP) in inducing protective immunity to malaria. VIP employs adeno associated virus (AAV) vectors to deliver genes encoding monoclonal antibodies (MAbs) to animals. Mice transduced by VIP vectors that encode monoclonal antibodies directed against the *P. falciparum* circumsporozoite protein (CSP) rapidly develop high serum levels of the MAb and are protected from experimental infection by a transgenic rodent parasite that expresses *P. falciparum* CSP. This project will assess in more depth the potential of VIP technology in malaria immunization. It has two specific aims: 1. to use the murine challenge model to identify additional MAbs with potential in the VIP system and optimize their expression *in vivo*, and 2. to test the most promising MAbs for protective efficacy in a non-human primate model of *P. falciparum* infection that employs *Aotus nancymaae* new-world monkeys.

2. Keywords: Malaria, monoclonal antibody, immunization, vaccine, vectored immunoprophylaxis, gene transfer, virus vector, adeno associated virus, AAV, *Plasmodium falciparum,* sporozoite, murine challenge model, non-human primate challenge model, *Aotus*

3. Accomplishments.

A. Major Goals	**Timeline (months)**	
	Projected	
Completed(%)		
Goal 1: VIP vector development		
1. Prepare, purify and sequence new MAbs	1-12	75
2. Construct first-round vectors	1-18	100
3. Optimize MAb expression in new vectors	3-18	100
Milestone: Selection of candidates for mouse experiments.	12-18	100
Goal 2: Evaluate candidate vectors in mice		
1. Local IRB/IACUC Approval	Completed	
2. Assess protection by VIP vectors; IV challenge	6-30	susp*.
3. Assess protection by VIP vectors; mosquito bite challenge	12-30	80
4. Determine mouse dose-responses; mosquito bite challenge	18-30	0
5. Assess protection by vector pairs; mosquito bite challenge	18-30	100
Milestones: Selection of VIP vectors for *Aotus* studies.	12, 18-24	100
Goal 3: Determine *Aotus* dose response	7-18	
1. Local IRB/IACUC Approval	Completed	
2. Dose response in *Aotus*	Completed	
Goal 4: *Aotus* challenge 1 (mAb 2A10)	13-30	0**

Goal 5: *Aotus* challenge 2 (mAbs TBD)	19-36	0**
Milestone: Selection of vectors potential clinical trials.	36	0**

* Suspended

** Please see **E.5. Challenges and Problems**, below

B. What was accomplished under these goals

Goal 1: VIP vector development*. 1. Prepare, purify and sequence new MAbs.* The amino acid sequence of one chain of a single previously-selected MAb (against CelTOS, see below) remains to be determined. This information is required for vector construction for this mAb. Work on this mAb has been suspended to expedite work on potent human anti-CSP mAbs whose sequences were reported in the literature [1,2]. If personnel become available the CelTOS effort may be re-activated; if it is, no difficulties are anticipated in completing it.

2. Construct first-round vectors. The recent publication of the amino acid sequences of a series of potent anti-CSP human mAbs (which eliminates the need for determination of sequence by us; [1,2]) has allowed us to reconsider candidates for murine and, potentially, non-human primate tests. In light of the availability of sequences, current plans include evaluation in the murine model of anti-CSP mAbs 2A10, 2C11, 5D5, 2H8, 667, CIS 43, and MGU12, the latter two from the recently-published literature. AAV8 vectors (for murine studies) have been prepared for all of these: 2A10 and 2C11 prior to initiation of the DoD project, 5D5, 2H8, and 667 during previous funding periods, and CIS43 and MGU12 during this funding period. One or two additional new human mAbs may be built using published amino acid sequence information if time and resources permit. Vectors encoding mAb directed against CelTOS, a protective antigen described by E. Angov of WRAIR remains to be built into a vector due to incomplete sequence data, as described above, but work on that mAb has been suspended.

3. Optimize MAb expression in new vectors. Vector-driven MAb expression is influenced by the amino acid sequence of the framework portions of the MAb variable regions. Alterations in the framework generally do not affect antibody binding, and so framework modifications can be used to modulate expression independently of antibody specificity and affinity. In an effort to maximize mAb expression from vectors encoding the new mAbs CIS43 and MGU12, framework sequences from our highest-expressing MAb (2A10) were incorporated into a vector that retains the specificity-determining regions of those antibodies. Disappointingly, mAb expression from the modified vectors was reduced compared to that from the original CIS43 and MGU12 vectors. Importantly, while it is clear from our published mouse data that high expression levels enhance protective efficacy, extravagant levels of expression of a potent MAb may not be needed to confer protection. Therefore, pursuit of enhanced MAb expression is not considered an essential element of the project and further optimization efforts will not be made under this award.

Goal 2: Evaluate candidate vectors in mice. *1. Assess protection by new VIP vectors; intravenous (IV) challenge.* During previous funding periods, three MAbs were assessed for protective efficacy by both IV injection of sporozoites and exposure to infected

mosquito bites. One is protective in both assays and one is protective in neither. The remaining MAb protects in mosquito bite challenge, but not IV challenge. Because mosquito bites represent the route of natural infection and seem from these results to provide a more sensitive indication of protection, use of IV challenge as a measure of efficacy for new vectors in mice will not be performed.

3. Assess protection by new VIP vectors; mosquito bite challenge. Mosquito bite challenge experiments have been completed in duplicate for 2A10, 2C11 (prior to this award), 5D5 (previous funding periods), and 2H8 and 667 (this funding period). Challenge experiments with the two new MAbs (CIS 43 and MGU12) are underway. Data for 2H8 and 667 are presented in Figure 1. Both of these mAbs, which are expressed at modest levels, provided protection in a proportion of immunized mice (50-70%) comparable to the highly-expressed 2A10 MAb whose characterization was completed prior to this award. Both of these MAbs therefore remain candidates for use in NHP studies.

4. Determine mouse dose-responses; mosquito bite challenge. No studies were conducted this funding period.

5. Assess protection by vector pairs; mosquito bite challenge. One study, which included the 2A10 MAb and MAb 5D5, was completed in the previous funding period. As reported in the 2017 Technical Progress Report, this pair was chosen because the two MAbs target distinct epitopes: the CSP central repeat (2A10) and a conserved epitope in CSP that lies near the site of a proteolytic cleavage that is required for cell invasion by sporozoites (5D5). 2A10 is protective in about 70% of animals, while 5D5 is not detectably protective alone. The combination had efficacy indistinguishable from that of 2A10 alone, indicating that in this case, no synergy occurs.

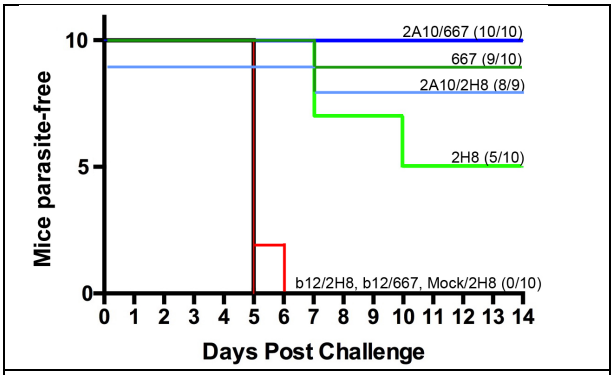
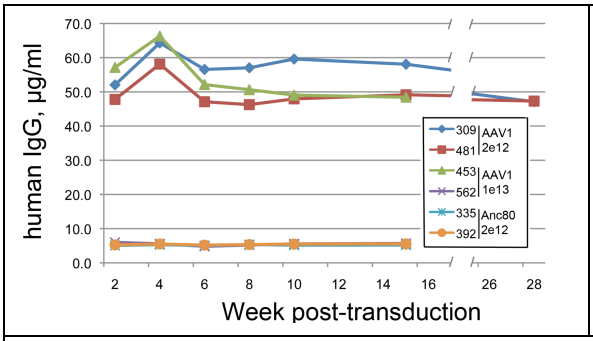


Figure 1. **Protection by mAbs 2H8 and 667 in mice.** AAV8 vectors encoding mAbs 2H8 and 667 (both anti-CSP), as well as positive (2A10) and negative (b12) control mAbs were each used to transduce 9 or 10 mice. Transduced mice were challenged by bites of mosquitoes infected with a transgenic *P. berghei* parasite that displays the *P. falciparum* CSP. Mice remaining parasite-free are plotted vs. day post-challenge. Mice that are parasite-free 14 days post-challenge are considered protected (noted for each vector). These experiments were done at different times, and positiv and negative controls for each therefore are included.

Goal 3: Determine *Aotus* dose response. 1. *Local IRB/IACUC and ACURO Approva*l has been obtained.

2. *Dose response in Aotus.* These studies were completed in the previous funding period with a malaria-irrelevant mAb (this was misreported in the 2017 report as anti-CSP mAb 2A10), using two doses based on literature values for related vectors. (The irrelevant mAb, against HIV gp120, was used in anticipation of subsequent use of these

animals in challenge studies.) Three of the four transduced animals produced the mAb, while one did not (Figure 2, left panel). Unexpectedly, the lower dose tested (2 x 10^{12} genome copies [GC] per monkey) proved to yield serum MAb levels equal to that of the higher dose (10^{13} GC/monkey) in responding monkeys. Thus, the system seems to be saturated with respect to the inoculum of AAV at these doses. Ultimately, it may be desirable to test lower doses to determine the minimum amounts of vector that produces a protective response in preparation for clinical trials. However, that must await the successful development of the challenge system (see below).



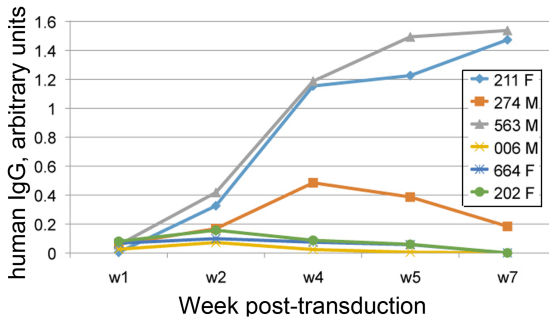


Figure 2. mAb expression after transduction of *Aotus*. Animals were transduced with AAV1-b12 (left) or AAV1-2A10 (right) at the indicated doses (left) or 10^{13} GC per animal (right). Human IgG expression was determined by ELISA. 'Anc80' refers to an AAV type used in two monkeys (left; see text)

An additional vector, with a capsid based on *in vitro* analyses of AAV capsid genes and projected to be insensitive antibodies to existing AAV types, was included in this study but was ineffective in producing mAb.

During this funding period, six additional monkeys have been transduced with vectors expressing mAb 2A10. Early expression data for those animals is presented in Figure 2, right panel. Three of these animals produced mAb and three did not; of the three positive animals, two exhibit stable expression while expression in the third is waning.

Our experience to date with a total of 12 *Aotus* has been that only about half of transduced animals produce antibody. The reason for this is not clear. All animals are screened and confirmed to be negative for AAV1 neutralizing antibody prior to purchase, ruling out pre-existing humoral immunity to AAV1. A 'take' in half of transduced animals is not an insurmountable difficulty in challenge experiments, although it will increase the number of animals that will be required to demonstrate efficacy. It would, of course, be unacceptable in immunization in humans. The basis of the phenomenon therefore should be explored in extensions of this project.

Goal 4: *Aotus* challenge 1 (mAb 2A10). As detailed in Section 5, below, difficulties encountered in implementing the challenge in the *Aotus* monkeys have prevented initiation of challenge experiments. These difficulties and progress in overcoming them are described in detail in Section 5.

Goal 5: *Aotus* challenge 2 (mAbs TBD). Not yet underway.

C. Opportunities for training and professional development. One Master's degree student and one postdoctoral fellow received training under this award during this funding period. The postdoctoral fellow has left the laboratory for a position in industry. The student will continue on the project.

D. How results were disseminated. Nothing to report

E. Plans for next reporting period

1. Murine challenge experiments will be completed for the remaining existing vectors (CIS43 and MGU12).

2. The alternative parasite strain described below will be tested for ability to produce sporozoites that efficiently infect *Aotus*. If reliable infection is achieved, challenges of the transduced animals on hand will be performed and estimates of protective efficacy might be obtained

4. Impact. Nothing to report.

5. Changes/Problems. A protocol for the assessment of pre-erythrocytic vaccine efficacy using sporozoite challenge in *Aotus nancymaae* monkeys has been published [3,4]. Briefly, splenectomized *Aotus* monkeys are challenged by IV injection of *Plasmodium falciparum* sporozoites, and are then monitored for development of parasitemia. Protection reduces the proportion of the challenged *Aotus* that become parasitemic. Published reports achieve a success rate of about 70% in infection of naive *Aotus* with the optimal parasite stain (Santa Lucia, see below). So far, despite extensive efforts, we have been unable to reproduce these results and this has precluded initiation of the next phase of the project, challenges in *Aotus* (Figure 3).

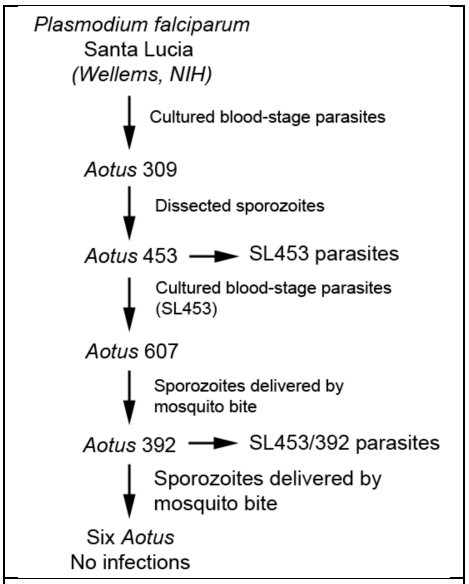


Figure 3. Passage history of *P. falciparum* Santa Lucia in *Aotus*

Few *P. falciparum* isolates are suitable for sporozoite infection of *Aotus* [3,4]. The isolate used in most of the published studies is Santa Lucia, a Honduran *P. falciparum* isolate. As reported in the 2017 Annual Technical Progress Report, the strain was located at the NIH and provided to us by Thomas Wellems. As received, St. Lucia grew in human RBCs in culture and gametocyte production could be induced by methods routinely used in the Johns Hopkins Malaria Research Institute (JHMRI) parasite core. *An stephensi* mosquitoes fed on the gametocyte cultures produced oocysts, but did not produce sporozoites, preventing sporozoite challenge.

It is not uncommon for malaria strains passed in culture to lose infectivity for mosquitoes, but infectious parasites can sometimes be selected from such populations by passage through animals. Therefore, two splenectomized *Aotus* (309, 481) were inoculated with blood-stage parasite cultures produced *in vitro*. Both *Aotus* became parasitemic and both developed gametocytes. *An stephensi* mosquitoes were fed on both monkeys. These mosquitoes developed oocysts and sporozoites, and multiple sporozoite preparations were made and injected IV into four naïve splenectomized *Aotus*. One of the four sporozoite-inoculated monkeys (453) developed parasitemia. Blood was drawn from this animal and aliquots were preserved. This

parasite (SL453), when amplified in culture, was infectious for mosquitoes and infected mosquitoes produce sporozoites, although in modest numbers. Thus, at least infectious sporozoite production was restored by animal passage.

Insufficient sporozoites for challenge experiments (50,000 per animal) could obtained from mosquitoes fed on SL453 blood differentiated *in vitro*. Therefore, banked 453 blood was used to infect another animal (670), which became parasitemic and was used to feed mosquitoes. Again, insufficient sporozoites were produced to permit IV inoculation. However, these mosquitoes were allowed to feed on two animals, one of which (392) became parasitemic. These parasites (SL453/392) thus were twice passaged through mosquitoes and to *Aotus via* sporozoite infection (once by injection and once by mosquito bite). Mosquitoes were fed on gametocytes produced *in vitro* from cultures infected with SL453/392 and became infected, but produced only modest sporozoite yields upon dissection. Therefore, six *Aotus* were exposed to these infected mosquitoes. After 60 days, none had become parasitemic and the experiment was terminated by pre-emptively treating all animals with chloroquine.

Our consistent inability to obtain high sporozoite yields in our mosquitoes (An. stephensi [repeatedly], An. gambiae [twice], and An. albopictus [once]), and the unreliability of transmission to *Aotus* by mosquito bites has forced us to conclude that using the published parasite, mosquito, and *Aotus* strains/species we will be unable to conduct the challenge experiments needed to evaluate our immunization approach.

A recent literature search found a single reference to use of a different *P. falciparum* strain, GB4, for infection of *Aotus*. We have obtained that strain, again from Thomas Wellems at the NIH. GB4 grows well in culture and on October 1, 2018, a blood culture was inoculated into a single *Aotus*. At the time of this writing, it is not known whether the animal is infected. Plans call for feeding mosquitoes on that animal when he becomes patent and feeding mosquitoes on blood cultures differentiated *in vitro*. Two naïve *Aotus* remain on hand, and if sporozoites are obtained from either monkey- or membrane-fed mosquitoes, those monkeys will be inoculated either IV or by mosquito bites, depending on sporozoite yields, to determine infectivity of GB4 sporozoites for *Aotus*. If good infectivity is seen, it remains possible that challenge of the six animals transduced with AAV1-2A10 (above) will yet shed light on the efficacy of antibody gene transfer in preventing malaria infection.

6. Products Nothing to report

7. Participants and collaborating Organizations.

Personnel

Gary Ketner Ph.D. No change

Robert J. Adams. DVM. No change

Gloria Shin, PhD. Postdoctoral Fellow. Anticipating the end of this award (August 31, 2018), Dr. Shin left the laboratory for a position in industry.

Suk Namkung, ScM student. Full time, no DoD support. Mr Namkung joined the laboratory in May, 2017 and will graduate in May, 2019.

Funding support: This award

Changes in active other support. Nothing to report

Organizations

PATH/MVI
2201 Westlake Avenue, Suite 200, Seattle, WA 98121
Furnished anti-CSP monoclonal antibody sequences

Walter Reed Army Institute of Research
503 Robert Grant Avenue
Silver Spring, MD 20910-7500
Furnished anti CelTOS monoclonal antibodies on a collaborative basis

Leidos
5202 Presidents Court
Frederick, MD 21703
Furnished 5D5 MAb sequence

8. Special reporting requirements. None

9. Appendices. None

References cited

1. Kisalu, N.K., A.H. Idris, C. Weidle, Y. Flores-Garcia, et al., *A human monoclonal antibody prevents malaria infection by targeting a new site of vulnerability on the parasite.* Nat Med, 2018. **24**: p. 408-416. PMC5893371.
2. Tan, J., B.K. Sack, D. Oyen, I. Zenklusen, et al., *A public antibody lineage that potently inhibits malaria infection through dual binding to the circumsporozoite protein.* Nat Med, 2018. **24**: p. 401-407. PMC5893353.
3. Collins, W.E., J.S. Sullivan, A. Williams, G.G. Galland, et al., *The Santa Lucia strain of Plasmodium falciparum in Aotus monkeys.* Am J Trop Med Hyg, 2009. **80**: p. 536-40.
4. Collins, W.E., J.S. Sullivan, A. Williams, D. Nace, et al., *Aotus nancymaae as a potential model for the testing of anti-sporozoite and liver stage vaccines against Plasmodium falciparum.* Am J Trop Med Hyg, 2006. **74**: p. 422-4.