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14. ABSTRACT. Malaria has proven refractory to conventional immunization approaches. This project explores a novel route to induction of anti-malarial immunity: adeno associated virus (AAV) vectored introduction of genes encoding known protective monoclonal antibodies (MAbs) into whole animals. Using a technology originally applied to expression of HIV antibodies [1], it was demonstrated that mice can be protected from *Plasmodium* infection by vector-driven expression of a monoclonal antibody (2A10) against circumsporozoite protein, an antigen found on the surface of the form of the parasite injected by mosquitoes [2]. Building on that observation, this project had two overall specific aims: 1. identification and evaluation of additional, potentially more effective, MAbs in the murine system, and 2. tests of protective efficacy of MAbs delivered by AAV vectors in a non-human primate (NHP; *Aotus nancymaae*) model of *P. falciparum* infection. Aim 1 has been accomplished. Progress has been made on Aim 2 including, critically, the demonstration that vector-delivered anti-malarial mAbs are capable of preventing liver invasion by *P. falciparum* sporozoites, the necessary first step in malaria infection. However, difficulties with replicating published malaria challenge protocols have hampered large-scale studies in NHPs and only a single mAb has been studied in *Aotus*. A new approach to efficacy studies in NHPs has been developed – assessment of liver burden as an indicator of infection – that will permit larger studies in the future that will address the unfinished elements of Aim 2.

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*, parasite liver burden, qPCR

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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 in both populations. However, extensive efforts to develop malaria vaccines using conventional approaches have been largely unsuccessful and no fully 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 has two specific aims: 1. Use the murine challenge model to identify additional MAbs with potential in the VIP system and optimize their expression *in vivo*, and 2. 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*, liver invasion, liver biopsy, bioluminescence

3. Accomplishments.

A. Major Goals	**Status**
Goal 1: VIP vector development	Completed
1. Prepare, purify and sequence new MAbs	
2. Construct first-round vectors	
3. Optimize MAb expression in new vectors	
Milestone: Selection of candidates for mouse experiments.	
Goal 2: Evaluate candidate vectors in mice	Completed
1. Local IRB/IACUC Approval	
2. Assess protection by VIP vectors; IV challenge	Discontinued
3. Assess protection by VIP vectors; mosquito bite challenge	
4. Determine mouse dose-responses; mosquito bite challenge	
5. Assess protection by vector pairs; mosquito bite challenge	
Milestones: Selection of VIP vectors for *Aotus* studies.	
Goal 3: Determine *Aotus* dose response	Completed
1. Local IRB/IACUC Approval	
2. Dose response in *Aotus*	

Goal 4: *Aotus* challenge 1 (mAb 2A10) Completed

Goal 5: *Aotus* challenge 2 (mAbs TBD) Will not be performed*

B. What was accomplished under these goals

Goal 1: VIP vector development. *1. Prepare, purify and sequence new mAbs.* It was initially anticipated that it would be necessary to determine the amino acid sequences of candidate mAbs in order to prepare synthetic mAb-encoding genes for incorporation into VIP vectors. The publication of the amino acid sequences of a series of potent anti-CSP human mAbs [3,4], sequences kindly furnished to us prior to publication by PATH-MVI, and a sequence provided by Leidos, has eliminated the need for determination of sequence by us.

2. Construct first-round vectors. Vectors encoding 10 distinct anti-CSP MAbs have been prepared (Table 1) seven of these have been fully tested in mice and one initially characterized in *Aotus (see below).*

mAb	Target	Efficacy (mice)	Ref.
2A10	CS repeat	Reference	[5]
2C11	CS repeat	<2A10	
5D5	CS junct.	Not protective	MVI
2H8	CS repeat	<2A10	[6]
667	CS repeat	~2A10	MVI
CIS43	CS junct.	>2A10	[3]
MGU12	CS junct .	>>2A10	[4]
668	CS repeat	Not yet tested	MVI
311	CS repeat		[7]
317	CS repeat		

Table 1 vectors prepared/under construction

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 *in vitro* 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 at this time is not considered an essential element of the project and further optimization efforts were not made. Anticipating use of VIP vectors in *Aotus*, it was also hypothesized that replacement of the human IgG1 and kappa constant regions in the mAb expression cassette might increase mAb accumulation in that system. Such 'Aotified' vectors have been prepared for mAb 2A10, but have not yet been evaluated *in vivo*.

Goal 2: Evaluate candidate vectors in mice. *1. Assess protection by new VIP vectors; intravenous (IV) challenge.* Because mosquito bite challenge (See below) has proven consistent and is the natural route of malaria infection, IV challenges were discontinued.

3. Assess protection by new VIP vectors; mosquito bite challenge. Mosquito bite challenge experiments have been completed for seven anti-CSP MAbs: 2A10, 2C11, 5D5, 2H8, 667, CIS 43 and MGU12. In these experiments, VIP vectors are packaged in AAV8 capsids, which have been shown to efficiently mediate VIP-driven mAb expression in mice. Mice are transduced by intramuscular injection of vector particles in saline. After mAb levels have reached a steady-state level (about 4 weeks post-transfection), transduced mice are challenged by bites of 5 – 10 mosquitoes infected with a transgenic *P. berghei* strain expressing *P. falciparum* CSP (*Pb/Pf*). Development of parasitemia in challenged mice is monitored by blood film microscopy. Figure 1 shows a Kaplan-Meier plot of survival (freedom from parasitemia) of mice transduced with 10^{11} genome copies of three mAbs (2A10, CIS43, and MGU12). Qualitative estimates of protective efficacy of each of the seven mAbs evaluated in mice, assessed by survival curves, are presented in Table 1. The MAb showing the highest protective efficacy in mice at the onset of this project, 2A10, was used as a standard for comparison. Three of the newly characterized MAbs are more effective in protecting mice from infection than 2A10, most strikingly MGU12. MGU 12 therefore is the current leading candidate for NHP studies when those are resumed.

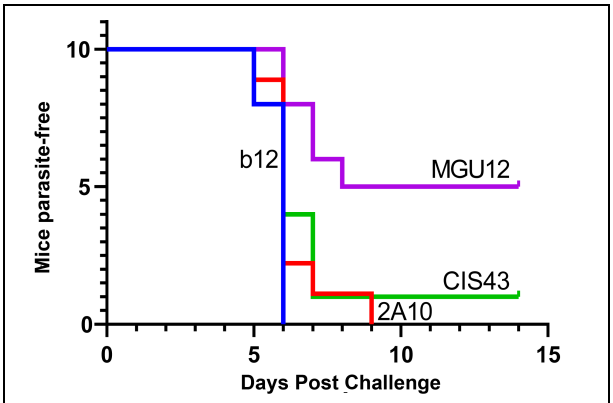

Figure 1. **Protection of mice by mAbs 2A10, CIS43, and MGU12.** AAV8 vectors encoding each mAb as well as a negative control mAb (b12) were used to transduce 9 or 10 mice. Transduced mice were challenged by bites of mosquitoes infected with a transgenic *P. berghei* parasite that displays the *P. falciparum* CSP. Mice remaining parasite-free are plotted vs. day post-challenge. Mice that are parasite-free 14 days post-challenge are considered protected. Against the parasite strain used in this experiment, protection by mAb 2A10 is modest. (Reproduced from the 2019 Annual Report.)

4. Determine mouse dose-responses (Figure 2). mAb expression in mice transduced with 10^{9} through 10^{11} genome copies of the mAb 2A10 vector was measured by ELISA of human IgG in mouse serum. In mice, serum mAb levels increased with increasing dose up to at least 10^{11} genome copies per mouse. Protection against infection in mosquito bite challenges also increased with dose.

5. Assess protection by vector pairs; mosquito bite challenge). One study, which examined mAbs 2A10 MAb and 5D5, was completed. This pair of mAbs 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). Thus, effects may be additive. 2A10 was protective in about 70% of animals in each of two repetitions of the experiment, while 5D5 is not detectably protective alone. The combination had

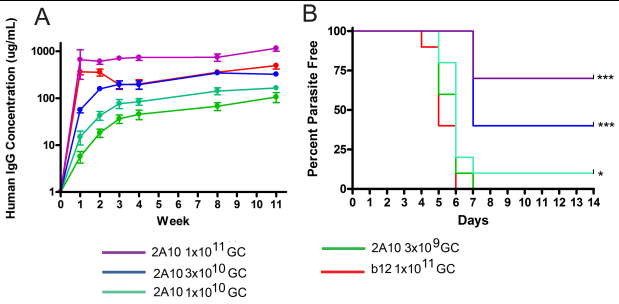

Figure 2. Dose responses in mice: Expression and protection from infection. (A) Expression of mAb 2A10 after transduction with varying vector doses. 2A10 levels were determined by human IgG ELISA using serum samples taken before and periodically after vector administration. Plot shows mean and standard error (n=10). (B) Mice were challenged 11 weeks after vector administration with *Pb/Pf* sporozoites by infected mosquito bite. Survival curves show the percentage of mice that do not show blood-stage parasites at each time point. ***p<0.005 *p<0.05.

efficacy indistinguishable from that of 2A10 alone, indicating that in this case, no synergy occurs (data not shown).

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

To investigate VIP in a system that will better reflect its behavior in humans, we have conducted studies in a well-recognized non-human primate model for human malaria infection, *Aotus nancymaae* monkeys. Use of VIP in non-human primates has not been well explored, although durable expression of antibody-like and therapeutic transgene products from AAV-based vectors has been reported in macaques and humans [8,9]. Our initial studies therefore were directed primarily at characterizing mAb expression from VIP vectors in *A. nancymaae.* Monkeys used in our studies were obtained from the Owl Monkey Breeding and Research Resource (OMBRR) facility at M. D. Anderson Cancer Center. All animals were negative for AAV1 neutralizing antibody. Because of their anticipated use in malaria challenges, all *Aotus* were splenectomized upon arrival (except 831 and 838, see discussion below), as required for the published sporozoite challenge protocol. All studies in monkeys were performed using VIP vectors packaged in AAV1 capsids, which efficiently mediate VIP-driven mAb expression in non-human primates. Transduction was by intramuscular injection of purified virus into the quadriceps at four to six sites (two or three in each leg). Plasma was obtained periodically and mAb expression was assessed by ELISA measurement of human IgG or anti-CSP activity. A summary of studies in *Aotus* is presented in Table 2.

2. *Dose response in Aotus.* (Figure 2). For the dose-response experiment, two *Aotus* each were transduced with the malaria-irrelevant mAb b12 at doses based on literature values for related vectors: 2 x 10^{12} genome copies [GC] per monkey and 10^{13} GC/monkey. (b12 was selected with the possibility in mind that these animals could be used as controls in subsequent challenge experiments.) On a GC/weight basis, these doses are comparable to the highest two doses used in the murine dose-response experiment described above. Three of the four transduced animals produced the mAb, while one did not (Figure 2, top 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. Therefore, 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.

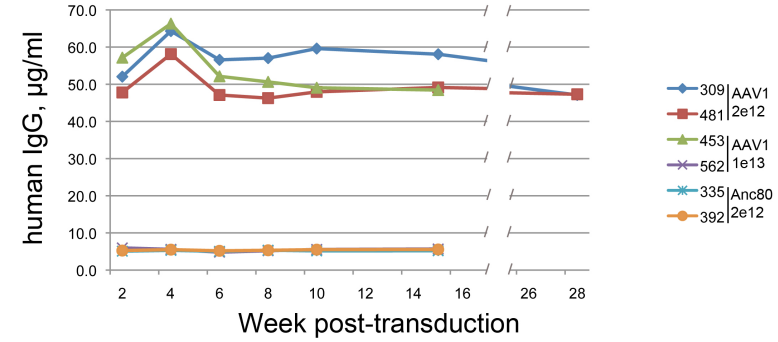

Figure 2. *Aotus* dose-response. *A. nancymaae* (individual animals are indicaed by 3-digit numbers) were transduced with AAV1-b12 at the indicated doses. Human IgG expression was measured by ELISA. 'Anc80' refers to a novel AAV capsid type used in two monkeys (see text).

An additional vector, Anc80, 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.

Grp	Aotus	VIP Vector	Target Ag.	Dose	CS peak recip. titer	Peak hIgG titer (µg/ml)	Duration (to week)	Spz. Challenge	Liver biopsy *Pf* 18S RNA
1	309	**b12/1**	gp120	2e12	-	65	>28	No	
1	481	**b12/1**	gp120	2e12	-	75	>28	No	
1	453	**b12/1**	gp120	1e13	-	65	>28	No	
1	562	**b12/1**	gp120	1e13	-	ND		No	
1	335	b12/anc	gp120	2e12	-	ND		No	
1	392	b12/anc	gp120	2e12	-	ND		No	
2	304	**2A10/1**	CS	4e12	>243,000		>17	No	
2	336	**2A10/1**	CS	4e12	9,000		Transient	No	
2	247	2A10/8	CS	4e12	500		Transient	No	
2	312	2A10/8	CS	4e12	500		Transient	No	
2	247*	2A10/7	CS	4e12	nd			No	
2	312*	2A10/7	CS	4e12	nd			No	
3	006	**2A10/1**	CS	4e12	2,700	nd	Transient	1×10^6	Positive
3	211	**2A10/1**	CS	4e12	210,000	794	>60	1×10^6	Negative
3	202	**2A10/1**	CS	4e12	2,700	nd	Transient	1×10^6	-
3	274	**2A10/1**	CS	4e12	12,000	167	Transient	1×10^6	Positive
3	563	**2A10/1**	CS	4e12	210,000	673	>60	1×10^6	Negative
3	664	**2A10/1**	CS	4e12	900	nd	Transient	1×10^6	Negative
3	666	None	-	-	-	-	-	1×10^6	Negative
4	130	None	-	-	-	-	-	3×10^6	Positive
4	663	None	-	-	-	-	-	3×10^6	Positive
5	831	None	-	-	-	-	-	1.2×10^6	See text
5	838	None	-	-	-	-	-	1.2×10^6	

Table 1. Transduction and challenges in *Aotus*. ND: None detected**.**

Goal 4: *Aotus* challenge 1 (mAb 2A10).

1. Development of a liver burden assay for infection. The proposed approach to measurement of protective efficacy by VIP-derived mAb employed challenge of transduced and naïve *Aotus nancymaae* monkeys by intravenous (IV) injection of *Plasmodium falciparum* sporozoites (spz), with infection assessed by progression to blood-stage parasitemia. *A. nancymaae* and other monkeys of the *Aotus* genus have been reported to be sensitive to infection by that method if specific strains of *P. falciparum* are used [11, 12}. We confirmed that *P. falciparum* grows well in *Aotus* if inoculated as blood-stage parasites, and that occasional spz infections occur. However, extensive attempts to achieve reliable spz infection using the published conditions were not successful. Unsuccessful attempts to achieve reproducible infection included use of three distinct parasite strains, three species of mosquitoes in spz preparation, IV and mosquito bite inoculation, and development of a line of the published parasite strain (St. Lucia) that had been passed twice through *Aotus* by spz inoculation. Thus the published procedure, at least in our hands, is unsuitable for assessing the efficacy of pre-erythrocytic malaria prophylaxis.

As an alternative to progression to patency as an endpoint, we have developed a method for quantifying of parasites in the liver after sporozoite injection as a measure of infection and, potentially, protection. In natural infection, after inoculation by the mosquito malaria sporozoites travel to the liver, where they develop for about 10 days to produce blood-stage merozoites, which go on to replicate in red blood cells. Our prophylaxis is intended to prevent liver infection by neutralizing sporozoites prior to their arrival there, and if successful, should reduce the number of parasites that can be detected in the liver (liver burden). Measurement of sporozoite RNA in liver is routinely used in mice to assess protective efficacy of pre-erythrocytic malaria

vaccines [10]. In a natural infection, very few sporozoites are injected and reach the liver, and it would likely not be possible to detect them reliably. However, challenge with a large enough dose of sporozoites may give rise to measureable liver burdens (this is routinely done in mice), and we have explored this possibility in *Aotus*.

In these experiments, we use the NF54 laboratory strain of *P. falciparum* as a source of sporozoites. We have shown that NF54 is not capable of progressing to blood stage disease in *Aotus*, but it produces plentiful sporozoites (20,000 or more per mosquito). This enables us to challenge with vast numbers of sporozoites (10^6 or more) in each animal, and calculations indicated that at this level of inoculation, parasites in the liver would be easily detectable by qPCR, accounting even for substantial losses in infectivity during sporozoite preparation. To determine whether liver burden measurement could indeed detect *P. falciparum* infection in *Aotus*, pre-challenge liver biopsies of ~100mg were obtained by laparotomy from two untransduced *Aotus* (Group 5, Table 2). After recovery from surgery, the monkeys were challenged by IV injection of 3 million sporozoites of the *P. falciparum* strain NF54 dissected from infected *An. stephensi* mosquitoes. 5 days post-infection, a second laparotomy was done to obtain post-challenge liver biopsies, again of about 100mg. Total RNA was isolated from the pre- and post-challenge biopsies. *P. falciparum* 18S RNA sequences were quantified by qRT-PCR, using *Aotus* β-actin mRNA as an internal control. Parasite 18S RNA was readily detected in both animals after challenge, and was absent from pre-challenge controls (Table 2). We conclude from this data that parasite liver RNA measurements are suitable for detecting liver invasion and thus for assessing the efficacy of antibody-mediated pre-erythrocytic immunity in *Aotus*. Following challenge, these animals were followed for 45 days post-challenge to determine whether NF54 eventually induces parasitemia. Consistent with our previous experience with NF54 and published reports [11], blood-stage parasites were not detected in challenged animals. At the end of that period, the monkeys were treated pre-emptively with chloroquine and retired.

2. Transduction and assessment of protection. Six monkeys (Figure 3; Group 3, Table 2) were transduced with 4 x 10^{12} GC of AAV1 vectors expressing mAb 2A10. Expression data for those animals is presented in Figure 3.

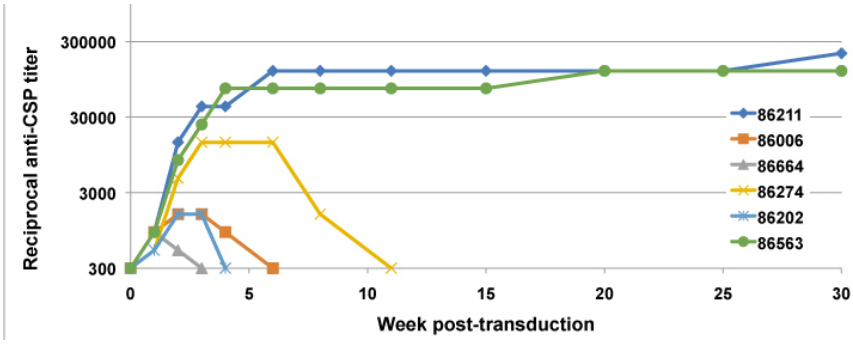

Figure 3. mAb 2A10 expression in *Aotus* Group 3 animals.

Two of the animals display persistent high-level expression of MAb2A10, while four expressed the MAb only transiently. This is consistent with experience with *Aotus* transduced with b12 earlier (3 of 4 monkeys expressed b12; see Figure 2). The reason for this variable 'take' 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. Successful MAb expression in half of transduced animals is not an insurmountable difficulty in challenge experiments, although it would 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 will be explored in future work on this project.

At about 60 weeks post-transduction, five of the six AAV1-2A10 transduced *Aotus* in Group 3 and an additional un-transduced monkey were inoculated with 1 million NF54 sporozoites (the sixth transduced Group 3 animal had been euthanized previously for reasons not related to the study). Liver biopsies were obtained by laparotomy pre-challenge and on days four and five post-challenge. Total RNA was extracted from these liver samples and parasite 18S and *Aotus* β-actin RNAs were quantified by RT-qPCR. The results of the challenge are included in Table 2. Two of three transduced animals that had transiently produced the 2A10 mAb but were anti-CS antibody negative at the time of the challenge (274 and 006) had parasite 18S RNA levels in the liver comparable to those seen in the previous untransduced controls. Neither of the animals with persistent anti-CS antibodies possessed a detectable liver burden and thus appear to have been protected from sporozoite invasion of the liver. The untransduced animal challenged in this experiment showed no parasite liver burden, nor did one of the monkeys without detectable antibody.

In aggregate, 2 of 3 naive animals and 4 of 6 animals without antibodies (including naïve and transduced animals that had expressed the mAb transiently) in Groups 3 and 4 challenged with NF54 sporozoites harbored parasites in their livers. Each of the 2 monkeys with persistent expression of anti-CSP antibodies after transduction had no detectable parasite liver burdens post-challenge. These numbers are too small to reach statistical significance. Nevertheless, the trend in this experiment strongly suggests that anti-CS antibody induced by vectored mAb gene delivery is protective against liver invasion by *P. falciparum* sporozoites.

The rate of infection in antibody-negative animals assessed by liver burden (66%) is essentially identical to the infection rate given in the published reports that described infection of *Aotus* with the St. Lucia *P. falciparum* strain (70%, [12]). This rate permits statistically robust of vaccine efficacy with reasonable numbers of animals, as discussed in the original application. Additionally, there is no requirement that the parasites used in liver burden challenges be of specific strains as demanded in published methodology. Thus, the liver burden protocol permits analysis of vaccine efficacy against diverse parasites, including laboratory strains and patient isolates, an important capability in vaccine development.

3. Development of a bioluminescent liver invasion assay. While laparotomy biopsies are feasible for the liver burden measurements, they are expensive, time consuming, and subject the animals to discomfort and the risk of surgical complications. Therefore, we have explored the possibility of using a non-invasive bioluminescent approach similar to that used in mice for quantifying liver burden in *Aotus* in a single preliminary experiment.

In this experiment, two naïve *Aotus* were inoculated by IV injection 1.2×10^6 sporozoites of a transgenic derivative of NF54 that expresses the firefly luciferase (*Pf* luc [13]). On days 2, 4, 6 and 18 (male only) post-inoculation the monkeys were injected with luciferase substrate (D-luciferin, PerkinElmer. 100mg/kg). Immediately after injection, the anesthetized animals were placed in the chamber of an *In Vivo* Imaging System (IVIS) Spectrum camera (Perkin-Elmer) and imaged for 1-5 minutes. The images obtained are presented in Figure 4. On d6, a liver biopsy sample was obtained from each animal by laparotomy to make possible comparison of liver parasite burden assessed by RNA quantitation and assessed by bioluminescence. Those measurements are pending. Imaging of these animals will continue for another 30 days.

The image data seems to reflect replication of the parasite from undetectable levels (d2 post-inoculation) to substantial expression in one animal and low but detectable expression in the other at d6. Apparently, the size of the initial inoculum is not large enough to produce a detectable signal directly. This suggests that in liver biopsy experiments the observation that some spz-inoculated animals are negative may be explained by their failure to support sufficient parasite replication in the liver to yield detectable RNA. This may make bioluminescence a

more reliable way of detecting liver invasion. If a difference in parasite replication from monkey to monkey is responsible for the variable levels of parasite RNA and bioluminescence observed in different animals, elucidation of the mechanisms responsible may further increase the sensitivity of the approach. If these preliminary results are confirmed and if the bioluminescent and qRT-PCR data are concordant. bioluminescent imaging may provide a simple tool for evaluation of pre-erythrocytic immunity in *Aotus*, and substantially facilitate pre-clinical malaria vaccine development.

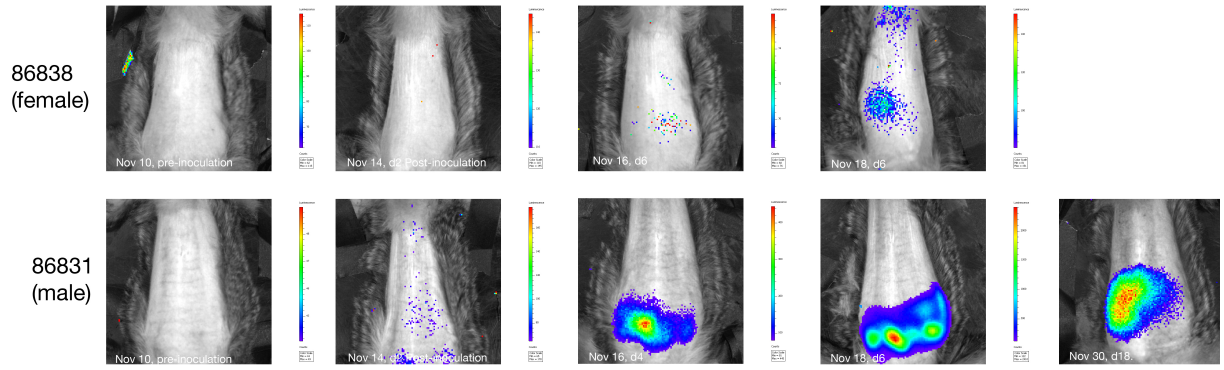

Figure 4. Bioluminescent images of *Pf* luc-inoculated *Aotus* monkeys Images were made prior to inoculation with *Pf* luc (left) and at days 2, 4, 6, and 18 (male only) post-inoculation. The torso is shown, from the rib cage downward. The animals are oriented with their heads at the bottom of the panel. Color key: blue – minimal bioluminescence – through red – maximal bioluminescence. Colorless – no luminescence detected.

Goal 5: *Aotus* challenge 2 (mAbs TBD). Time constraints prevented this work.

C. Opportunities for training and professional development. One Master's student and one postdoctoral fellow received training under this grant during this funding period.

D. How results were disseminated. A manuscript describing the transduction/challenge of Group 3 animals is in preparation. This manuscript will include the qRT-PCR liver burden methodology. A short manuscript describing the bioluminescent assay will be prepared, pending the qRT-PCR results on the liver biopsy samples in hand from that experiment.

E. Plans for next reporting period

Nothing to report.

4. Impact.

1. Impact on the major disciplines of the project. This work provides the first detailed information on AAV-mediated transfer of mAb genes to non-human primates (NHPs). This knowledge will inform further studies on use of VIP in malaria prophylaxis, and will similarly be applicable to evaluation of VIP against other pathogens in NHP model systems.

The development of a robust alternative approach to detecting infection in *Aotus* will additionally facilitate pre-clinical malaria vaccine studies, both using VIP other conventional methodologies.

2-4. Impacts on other disciplines, technology transfer, and society. Nothing to report.

1. **5. Changes/Problems.** The major obstacle to progress in this project was the failure to replicate the published protocol for malaria challenge in *Aotus*. Attempts to do so absorbed substantial time and financial resources, as laid out in previous Technical Reports. As detailed above, the published procedure for assessing infection in *Aotus* (progress to parasitemia) was abandoned and an alternative methodology (liver burden measurement) was developed. This solution permitted progress, although it came too late to permit full accomplishment of project goals.

Unexpected variability in the response to individual *Aotus* to transduction with VIP vectors also presented a problem that limited the statistical power of the study. This problem is not insurmountable, and investigations of its causes may also make it possible to avoid the problem in the future.

Neither of these issues required fundamental changes in the goals of the project.

6. Products Nothing to report

7. Participants and collaborating Organizations.

Personnel

Gary Ketner Ph.D. (P.I.) 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 an academic laboratory at Johns Hopkins.

Suk Namkung, ScM student. Full time, no DoD support. Mr Namkung joined the laboratory in May, 2017 graduated 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.

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