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<i>vivax</i> was not reversed in <i>Aot</i>	tus using prochlorperazine and c	hloroquine alone or in (combination during a
seven day regimen. An AMA-1	l erythrocytic DNA vaccine, part	tially protected 1/3 mor	ikeys, which showed
a low grade parasitemia that	t ended in treatment due to a	40% reduction in her	natocrit (Hto). After
rechallenge, all monkeys excep	ot for one that selfcured, had to	be treated 22-29 days	post inoculation (PI)
due to their low Htos. An EBA	-1 DNA erythrocytic vaccine off	fered partial protection	in 1/3 monkeys that
self cured 21 days Pl. Mt	iltiple rechallenges with the F	VO strain yielded ste	rile immunity. The
effectiveness of the intrader	mal (ID) route of inoculation V	s the IM route at ind	lucing high levels of
antibodies in <i>Aotus</i> was confi	rmed when a distinct antigenic I	ONA vaccine such as H	sBAg was used. The
addition of Uligonucleotides to	the vaccine formulation greatly	increased the antibody	/ responses observed
with this antigen. Cultured	Metloquine and Atovaquone:	Malarone resistant str	ains of <i>Plasmodium</i>
<i>Taiciparum</i> did not established	patent infections in Aotus nor 1	the <i>P. vivax</i> Sal 1 strai	in. <i>Aotus</i> immunized
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FOREWORD

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In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

Principal Investigator's Signature

Date

March 10, 1997.

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INTRODUCTION

Each year there are 300-500 million new infections and 2-5 million deaths attributable to malaria that occur primarily in countries in the tropics, particularly in sub-Saharan Africa. (4) During the past 10-20 years the malaria problem has intensified in some parts of the world because parasites have developed resistance to drugs used for treatment and prevention; the anopheles mosquito, which transmits the parasite to humans, has developed resistance to insecticides, and control efforts have been reduced as resources have diminished in some developing countries (5).

The use of Aotus lemurinus lemurinus (Panamanian Aotus monkey), cariotypes VIII and IX (11) as a model to study malaria drug resistance and vaccine efficacy, have been ongoing at Gorgas Memorial Laboratory since 1976, due in part to the availability of this monkey in Panama (15), and also to the increasing drug resistance exhibited by the highly pathogenic Plasmodium falciparum parasites in Asia, Africa, and Latin America, and more recently Plasmodium vivax in the Melanesian and Indonesian archipelago (16). Previously, Schmidt (21, 22) used the Colombian Aotus as the experimental host for antimalarial drug studies, but embargoes imposed by South American countries on the exportation of monkeys in the mid 1970's seriously restricted the use of Aotus for biomedical research in the United States, and in 1976 the project was transferred to Gorgas Memorial Laboratory where Panamanian Aotus were available for research. Since then, three strains of P. falciparum, Vietnam Smith, Uganda Palo Alto, and Vietnam Oak Knoll, had been adapted to Panamanian Aotus. These strains exhibit diverse susceptibility and/or resistance to standard antimalarial agents.

The course of untreated infections in Panamanian *Aotus* has been characterized and compared with that in *Aotus* of Colombia (20). Overall, the virulence of these strains was less in Panamanian than in Colombian owl monkeys, as indicated by lower mortality rates of Panamanian monkeys during the first 30 days of patency. Maximum parasitemias of the Vietnam Smith and Uganda Palo Alto strains were, however, significantly higher during the first 15 days of patency in Panamanian than in Colombian owl monkeys. These quantitative differences in infection parameters between Panamanian and Colombian owl monkeys have not invalidated the use of the former for evaluation of new antimalarial drugs.

Numerous candidate antimalarial drugs of diverse chemical classes have been evaluated against trophozoite-induced infections of one or more *P. falciparum* strains during the course of these contracts. In seeking ANNUAL REPORT/96-97 CONTRACT DAMD17-96-C-6051

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alternatives to primaquine, two 8-aminoquinolines proved to be active against the blood stages of *P. falciparum* (2, 13). Desferrioxamine, an ironspecific- chelating agent, was shown to suppress parasitemias of the virulent Uganda Palo Alto strain of *P. falciparum* (18). The *in vitro* activity of two halogenated histidine analogs was not confirmed by evaluation against *P. falciparum* infections in owl monkeys (17).

Chloroquine-resistance of *P. falciparum* represents the greatest challenge in developing effective antimalarial drugs. Reversal of chloroquine-resistance in *P. falciparum, in vitro*, was achieved by the co-administration of verapamil (a calcium channel blocker) plus chloroquine (12). Other in vitro studies have shown that there is a significantly greater efflux of chloroquine from erythrocytes containing falciparum parasites resistant to chloroquine than from red cells parasitized by chloroquine-sensitive falciparum malaria (9). Calcium channel blockers appear to prevent this active efflux of chloroquine, thus allowing the drug to accumulate to parasiticidal levels.

Based upon the success of *in vitro* reversal of chloroquine-resistance, trials were initiated to determine if resistance could be reversed in *Aotus* infected with the chloroquine-resistant Vietnam Smith strain of *P. falciparum*. Six calcium channel blockers, or similarly acting drugs, were co-administered with chloroquine in diverse regimens. The desideratum of chloroquine-resistance reversal was administration of a single course of treatment, with parasite clearance and infection cure. Suppression of parasitemia was obtained during an initial course of treatment, but parasite clearance and cure occurred in some instances only after re-treatment. Such infection parameters were similar to those in monkeys with self-limited infections and cure could be attributed to acquired immunity.

Limited trials with desipramine, Norpramin , a tricyclic psychotropic drug, demonstrated the feasibility of reversing chloroquine-resistance in vivo (1). parasite clearance was obtained, but the infection was not cured.

Subsequently, in vivo reversal of chloroquine resistance was obtained with combinations of chloroquine plus chlorpromazine or prochlorperazine. Such reversal was exhibited by rapid suppression and clearance of parasitemia, resulting in infection cure without retreatment **(10)**.

Evaluation of two oil-soluble derivatives of artemisinin, artemether and arteether, demonstrates that both possess similar activity to cure infections of a multi-drug resistant *P. falciparum* strain in *Aotus* (23).

Some strains of *P. vivax* from the Melanesian and Indonesian archipelago have demonstrated resistance to treatment with chloroquine (14, 19). Unlike chloroquine-resistant falciparum malaria, there exists no easy alternative to chloroquine-resistant strains of vivax malaria. Using WR 238605 alone or in combination with chloroquine in Panamanian *Aotus* monkeys it was demonstrated that WR238605 is an alternative treatment for chloroquine-resistant vivax malaria (16). The compound WR 238605 is a primaquine analog developed by the US Army as a better tolerated, more effective replacement for primaquine.

Both the purpose and methods of approach of the present work remains essentially unchanged since 1976, viz to ascertain the antimalarial activity of drugs against *P. falciparum* and *P. vivax* in *Aotus*. The method of approach may vary on an ad hoc basis, such as administering a combination of drugs.

The long term goal of the second part of this project is to develop fully protective plasmid DNA vaccines that induce protective immune responses against the sporozoite, liver and erythrocytic stages of *P. falciparum*. If successful, it will establish for the first time that plasmid DNA vaccines can protect non-human primates, a critical step forward using plasmid DNA vaccines in humans.

Vaccines are aimed at inducing immune responses that disrupt the complex cycle of the parasite at one more points: anti-sporozoite antibodies that prevent invasion of hepatocytes; cytotoxic T lymphocytes, cytokines, and antibodies that eliminate infected hepatocytes; antimerozoite antibodies that prevent invasion of erythrocytes; antibodies that neutralize parasite exoantigens known to induce harmful cytokine responses; antibodies that attack infected erythrocytes; cytokines that kill parasites within erythrocytes; and, anti-sexual stage antibodies that prevent the development of sporozoites in the mosquito.

Previous trials of malaria blood stage vaccine have shown that the Panamanian *Aotus P. falciparum* model to be suitable for this purpose. **(6-8)**.

Immunogenicity studies of a plasmid DNA vaccine encoding the circunsporozite *P. yoelli* rodent malaria gene (PyCSP) in Panamanian *Aotus* monkeys demonstrated that the intradermal route of inoculation (ID) induces a higher level of antibodies than the Intramuscular route (IM). Antibody levels induced in this manner reached a peak at week 9 and titers declined to 50% their peak value by week 14. When boosted at week 46 antibody levels increase 4 fold by week 49. This was comparable to

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antibodies generated with a Multiple Antigen synthetic peptide vaccine (MAP) delivered with an adjuvant **(4)**

The purpose of this report is to: 1) Present data on the evaluation of potential antimalarial activity of drugs in the pre-clinical model of *Aotus I. lemurinus* (Panamanian night monkey) experimentally infected with *P. falciparum* or *P. vivax*, and 2) data on plasmid DNA malaria vaccine experiments. These studies were supported by the U.S. Army and the U.S. Navy Malaria Programs.

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BODY

I. Experimental Methods

The first aim of this project is to evaluate the potential antimalarial activity of drugs, or combination thereof, in the preclinical model of *Aotus* experimentally infected with *P. falciparum* (or *P. vivax*). Specifically, the vertebrate host is *Aotus I. lemurinus*, the Panamanian night monkey. These animals are either feral, laboratory adapted or laboratory born. No naturally acquired, human plasmodium infection has been reported in *Aotus*. The Vietnam Smith/RE strain of *P. falciparum* was adapted to *Aotus* of Colombian origin in 1971 (21) and in Panamanian *Aotus* in 1976. (20). The course of untreated infections, essential for comparison with treated infections, has been documented in Panamanian *Aotus* (20). This plasmodium strain is resistant to maximally tolerated doses of chloroquine, pyrimethamine, and quinine (22).

To initiate an experiment, infected blood (with 2.5% sodium citrate as the anticoagulant) from an untreated *Aotus* was diluted appropriately in chilled saline (0.85%), such that each milliliter contained 5,000,000 parasites. This amount was inoculated into the saphenous vein of experimental and control monkeys.

Blood films, prepared and examined daily beginning on the first postinoculation day, were stained with Giemsa. Parasitemias were evaluated as follows: negative, if no parasites were detected on a thick blood film after examination for at least 5 minutes; <10 parasites per cmm, if positive only on the thick blood film; parasite enumeration was by the Earle-Perez method and reported as the number of parasites per cmm. **(3)**

Blood films from untreated *Aotus*, serving as passage and/or control subjects, were prepared and examined daily during the primary patent period, and daily thereafter for at least three consecutive days after parasites could last be detected on thick blood films. When parasitemia had cleared, films were made and examined twice weekly until a total of 100 negative days had been recorded. If recrudescence occurred, blood films were obtained again on a daily basis.

Parasitemias were evaluated daily during the treatment period and until blood films were negative for at least seven consecutive days. The frequency of smearing was then reduced to two times per week (Monday and Thursdays or Tuesdays and Fridays). If no recrudescences occurred during a 100 day examination period, the infection was considered to have been cured. Drug doses were calculated as mg base per kg of body weight. Stock solutions of water soluble compounds, at appropriate concentrations, were prepared with distilled water and stored at 8° C for the treatment period. If a compound was water insoluble, a suspension of the requisite amount of drug was prepared daily with 0.3% methylcellulose (in distilled water).

Oral administration of drugs was by gastric intubation with a 14 French catheter. The total volume of fluid administered, drug solution or suspension, and rinse was 14 ml.

Response to treatment was categorized as clearance and cure, clearance and recrudescence, or suppression without clearance. The day of clearance was defined as the first of three consecutive days in which the thick blood films were parasite negative. The day of recrudescence was the first of three consecutive days of positive thick blood films after a period of clearance. Suppression was defined as a transient decrease in the parasite count post-treatment without clearance.

The second purpose of this project is to ultimately evaluate recombinant vaccines against the blood and sporozoite stages of *P. falciparum* and against the blood stages of *P. vivax* in the Panamanian *Aotus* model. Prior to actual anti-parasitic experiments various routes of administration of a candidate vaccine must be tried so as to produce significant antibody levels. These trials will be detailed in the appropriate sections, as will other experiments associated with the Navy Malaria program.

II. Results

A. Passage of *P. falciparum* Smith/RE strain

In order to bring up a frozen strain of Smith/RE *P. falciparum,* two malaria naive monkeys were inoculated intraperitoneally (IP) with blood from two different donor monkeys on 28 August 1996. Both animals remained negative for more than sixty-four days.

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B. Reversal of Chloroquine resistance of *P. vivax* AMRU-1 strain.

Previous studies with a CQR *P. falciparum* have shown that it is possible to achieve *in vivo* reversal of CQR by the co-administration of prochlorperazine and chloroquine, as evidenced by infection cure. Neither drug alone affects such cure (10).

This study was designed to determine if CQR of the AMRU-1 strain (P. vivax) can be reversed in vivo by prochlorperazine plus chloroquine.

On 21 October 1996, each of 10 Aotus I. lemurinus, cured of P. *falciparum*, was inoculated intravenously with 5 x 10^6 AMRU-1 strain parasites of P. vivax, and divided into three groups of three monkeys plus a single untreated control to determine if the co-administration of prochlorperazine (WR 280003 AC; BN 43106) and chloroquine (WR 1544 BM; AR 20613) against infections of the AMRU-1 strain (CQR) of P. vivax will reverse chloroquine resistance. As shown in Table 1, Prochlorperazine alone at 20 mg/kg x 7 days did not have any effect on 3/3 animals from Group 1. Animals from this group cleared 18 and 37 days post inoculation (PI). One animal of this group died of malaria 20 days PI and the animal which cleared 18 days PI had a transient two days recrudescence 4 days after clearance. The two surviving animals remained negative for more than 61 and 74 days respectively. Group 2, that received Prochlorperazine 20 mg/kg plus chloroquine 10.0 mg/kg cleared their parasitemias 4-7 days Pl without recrudescence for more than 87-89 days. In group 3, that received Chloroquine 10.0 mg/kg 2/3 monkeys cleared parasitemias 3-8 days Pl without recrudescence remaining negative for more than 84-88 days Pl. Although animals from this group, one died of malaria 8 days after inoculation.

A striking finding during the course of this experiment was the anemia related deaths observed in two monkeys and that three had to be transfused with fresh whole blood due to their extremely low hematocrits. It is postulated from these findings that another cause different than *P. vivax* AMRU-1 infection might have been the cause of death in these animals.

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In vivo reversal of CQR of the AMRU-1 strain by the co-administration of prochlorperazine could not **be definitively demonstrated** with a 7 day course treatment in this experiment.

C. Adaptation of *in vitro* cultured Mefloquine and Atovaquone:Malarone resistant strains of *P. falciparum* to Aotus monkeys.

In an attempt to adapt *in vitro* cultures of a Mefloquine (Mef 2.5) and an Atovaquone (C2B) resistant strains of *P. falciparum*, two malaria naive splenectomized monkeys were inoculated intravenously (IV) on 27 January 1997, with 2 mls of packed red cells from room temperature *in vitro* culture parasites. No parasites were detected in daily blood smears for more than 42 days Pl.

D. Passage of P. vivax AMRU-1 strain.

On 15 October 1996, one monkey was inoculated intraperitoneally (IP) for passage of a frozen strain of AMRU-1 *P. vivax* malaria. The monkey never developed a detectable parasitemia and remained negative for more than 75 days PI.

E. Passage of *P. vivax* Sal-1 strain.

To bring up a frozen strain of Sal-1 *P. vivax*, two *P. falciparum* cured monkeys, one intact and one splenectomized, were inoculated IP on 2 and 18 October 1996. Both animals remained negative for more than 118 and 121 days respectively.

F. Efficacy of a *P. falciparum* AMA-1 Erythrocytic DNA vaccine in Aotus monkeys.

Nine malaria naive *Aotus* monkeys divided into 3 groups of 3 monkeys, were vaccinated intradermally with four doses of a plasmid DNA encoding AMA-1 with or without lipid MPL. They were challenged with 1 x 10^5 parasites of the *P. falciparum* FVO strain on 19 September, 1996. All vaccinated and control animals were patent by day 7 PI with a prepatent period ranging from 3-6 days as shown in table 2. Control animals were treated on day 12 PI and treatment was initiated in all vaccinated animals between days 13-15 PI. Except for one animal of Group 1 (Monkey 12770)

which maintained parasitemia levels under 150,000 parasites/ul, all of the remaining animals had steadily increasing parasitemias that reached the 300,000 parasites/ul treatment threshold. However, its hematocrit had a 40% reduction during the course of parasitemia and had to be treated with mefloquine. During the course of this experiment two monkeys died. One due to aspiration pneumonia during oral mefloquine treatment and another (12788) to malaria, 39 days PI.

On January 7, 1997 all of the remaining monkeys were re-challenged with 10,000 parasites of a *P. falciparum* FVO strain. This time, as shown in Table 3, infection in all monkeys were patent between days 7-8 Pl. Parasitemias were below 100,000 parasites/ul, but their hematocrits suffered a significant reduction by day 22 Pl, when two animals 12770 and 12792 had to be treated with mefloquine. By day 24 Pl, three other monkeys 12790, 12791 and 12793 had to be treated as well. Albeit, monkey 12787 from Group 1 and 12789 from Group 2 had a parasitemia course below 10 parasites/ul, the former had to be treated 29 days Pl and the latter self cured.

G. Efficacy of *P. falciparum* EBA-175 DNA vaccine in *Aotus* monkeys.

To test the efficacy of *P. falciparum* EBA-175 erythrocytic plasmid DNA vaccine, nine naive *Aotus* were divided into three groups of 3 monkeys and vaccinated intradermally with 500 ug of plasmid encoding the EBA-175 and P2P30 tetanus toxin protein repeat. On 7 January 1997, all animals received 1 x 10^5 parasites of the FVO strain of *P. falciparum*. As seen in Table 4, by day 6 PI all had patent infections. Treatment with mefloquine was initiated between 11-15 days PI in all animals, except for monkey 12811 in Group 2 and control animal 12813 which by that time had not reached the 300,000 parasites/ml mark. However, by day 20 the hematocrit of monkey 12813 was 20% and had to be transfused with whole blood. This animal died the next day. Monkey 12811 which Hto remained over 30% during the course of infection, self cured 21 days PI.

H. Immunogenicity of a PfCSP MAP Vaccine in *Aotus*

Linear and Multiple Antigen Peptides (MAP) sequences derived from the PfCSP protein of *P. falciparum*, were synthesized as peptide sequences with an exogenous T-cell helper epitope (P2P30 or PADRE). These synthetic peptide sequences were incorporated into a liposome vaccine formulation and delivered IM with Alum. The purpose of this experiment

was to test the relative immunogenicity of these vaccine candidates in a primate model.

On January 9, 1997, thirty *P. falciparum* and *vivax* double cured Aotus monkeys were divided into six groups of 6 monkeys each and vaccinated with synthetic peptides derived from the PfCSP sequence in different peptide/helper formulations with monophosphoryl lipid A. Each monkey was inoculated IM in the the quadriceps muscle, with 400 ul total volume; (200 ul/site). All animals received 100 ug of antigen per dose and will be immunized three times at monthly intervals. Serum collection for antibody determinations will be carried out every two weeks until 26 June 1997. No parasite challenge will be carried out in this experiment. This experiment is still on progress by the time of this report.

I. Induction of immunity by repeated challenge with the FVO strain of *P. falciparum*

Of the various *P. falciparum* strains adapted to nonhuman primates, the FVO (Vietnam-Oak Knoll) strain would be useful for vaccine studies as only 25-30% of infected Panamanian *Aotus* self-cure (20). The remainder of the infected animals require curative drug treatment or death will ensue. When evaluating a vaccine, the higher the proportion of self-cure, the greater the number of animals needed in each experimental group to assure that the animals are protected by the vaccine and not selfcuring.

To compare the efficacy of an "artificial" vaccine with protection afforded by acquired immunity, an experiment was initiated to induce immunity by repeated trophozoite challenge. Initial results were given in the previous report. Briefly, malaria naive Panamanian *Aotus* were inoculated with 10,000 parasites of the FVO strain, the parasitemia monitored daily by blood film examination, and the infection cured with mefloquine (40.0 mg/kg, oral, x 3 days) when parasitemia approximated 800,000 per cmm. About 4 to 6 weeks after infection cure, the animals will be rechallenged with parasites from a donor monkey whose infection was initiated by cryopreserved parasites. Donor animals, cured of infection, were recycled into the challenge group. Challenges will be repeated until the monkeys demonstrate complete immunity.

The current results summarized in Table 7 indicate that sterile immunity has been induced in twelve monkeys following 2, 3 or 4 rechallenges, being the last one on September 19, 1996. Following this homologous rechallenge, a heterologous challenge is planned with a plasmodium strain yet to be determined.

J. Immunogenicity studies of a MAP vs Linear NANP vs NANPNVDP Malaria peptide vaccine in *Aotus*.

On 5 August 1996 a total of 18 malaria double cured *Aotus I. lemurinus* monkeys were divided into 6 groups of 3 monkeys each and immunized IM in the bilateral quadriceps (200 ul each) with a dose of 100 ug in 400 ul of a Peptide vaccine formulation as follows:

Group 1 monkeys were immunized with a Linear (NANP)6 P2P30 peptide. Group 2 with a Linear (NANPNVDP)3 P2P30 peptide. Group 3. Group 4 with an MAP4 with an MAP4 (NANP)6 P2P30 peptide. PADRE-PFB 5 with а Group P2P30 peptide. (NANPNVDP)3 (aKXVAAWTLKAa(NANP)4-GGS) peptide and Group 6 was inoculated with alum as a Control. All animal were inmunized three times and bled five times at monthly intervals. No challenge was carried out in this experiment and it was completed on 20 December 1996. of the Results immunogenicity studies are pending.

K. DNA-based immunization of *Aotus* against HBsAg

In order to elucidate why the IM route using a PyCSP malaria DNA vaccine was not effective in Aotus as has been previously reported (4), a HBsAg hepatitis DNA vaccine known to be immunogenic by the IM route in Macaca mullata monkeys, was chosen as an antigenically distinct vaccine. Forty P. falciparum and vivax double cured Aotus known to be negative to HBsAg hepatitis antibodies, were divided into 10 groups of 4 monkeys each, and vaccinated using either the IM, ID or Intranasal routes. Vaccine formulations consisted of saline, liposome and oligonucleotides or a The positive control group was combination of one or all of them. vaccinated with a commercial recombinant HBsAg protein vaccine. All monkeys were bled 7 times for HBsAg antibody level determination and three times for lymphocyte collection which were used in cellular immunity In addition, on 27 September, 1996 all animals received a studies. Immunogenicity studies are in HBsAg protein booster. recombinant progress. This experiment ended on 20 December 1996. The addition of oligonucleotides to the vaccine formulation greatly increased the antibody responses observed with this antigen.

L. DNA Immunization with CSP, SSP2 and Exp-1 *P. falciparum* pre-erytrocytic vaccine and challenge.

On July 17, 1996, 28 malaria naive lab-born monkeys, previously vaccinated with 4 doses of a CSP, SSP2, and EXP-1 plasmid DNA preerythrocytic vaccine, were challenged with 21,300 sporozoites of the Santa Lucia strain of *P. falciparum*. All monkeys were splenectomized 14,15 and 16 days later and tissue samples, tissue impression smears and samples for PCR were collected. Daily thick blood films, taken for more than sixty days, were negative. In addition bi-weekly blood sampling for PCR malaria detection were also negative. Spleen impression smears taken during splenectomies did not reveal any parasites.

Due to technical difficuties at obtaining a readily source of sporozoites, a new challenge had to be postponed until further notice.

III. Conclusions

Since results of the coadministration of prochlorperazine (WR 280003 AC; BN 43106) at 20 mg/kg with chloroquine (WR 1544 BM; AR 20613) at 10 mg/kg x 7 days were inconclusive, an experiment is **planned to** evaluate (WR 280003 AC; BN 43106) at 20 mg/kg with chloroquine (WR 1544 BM; AR 20613) at 10 mg/kg x 3 days only, against AMRU-1 strain infections of *P. vivax*.

Results of the challenge experiments of *Aotus* vaccinated with plasmid DNA vaccines coding for the AMA-1 and EBA-175 genes, showed that 1/3 monkeys were partially protected and self cured against challenge of *P. falciparum* (Vietnam-Oak Knoll strain). This results will have to be weighted against a known 20-30% self cure rate for the FVO strain of *P. falciparum* in *Aotus* monkeys, with a larger experiment with sufficient number of animals to ascertain statistical significance.

Results of the inoculation of Panamanian *Aotus* vaccinated with a pre-erytrocytic plasmid DNA vaccine containing CSP, SSP2 and Exp-1 genes of *P. falciparum*, with sporozoites of a the Santa Lucia were inconclusive. Due to the fact that, after 64 days PI, patent infection was not achieved. A new challenge is expected to occur when sporozoites are made available.

Homologous re-challenge with Vietnam-Oak Knoll parasites has, to date, resulted in twelve *Aotus* with sterile immunity. These animals, as well as others without such immunity will be re-challenge both with a heterologous strain. Data will be compared with a hopefully effective DNA vaccine.

The absence or low antibody responses observed in previous experiments with a PyCSP DNA vaccine when *Aotus* were vaccinated by the IM route was confirmed when a distinct antigenic DNA vaccine as a Hepatitis **HBsAg**, know to induce antibody levels in other primate species was used. A striking finding during the course of this experiment was that the co-administration of oligos, induced a high antibody response not previously seen when an equivalent dose of a PyCSP DNA vaccine was used. Future experiments will be carried out in order to compare the effectiveness of oligos at inducing high antibody responses when combined with a DNA vaccine using different doses and routes of inoculation.

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TABLE 1.

DETAILED ACTIVITY OF PROCHLORPERAZINE WR280003AC ALONE OR IN COMBINATION WITH WR 1544 BM CHLOROQUINE AGAINST INFECTIONS OF THE AMRU-1 STRAIN (COR) OF *PLASMODIUM VIVAX* IN *AOTUS*.

							PARASIT	EMIA PE	R cmm	X 10 ³					
AOTUS NO.	DAY PAT.	MG/KG DOSE	DAY PRE RX	-	DAY 2	3 3	TREAT 4	MENT 5	9	~	. .	DAYS 2	POST 3	TREA 4	TMENT DAYS NEG.
12651	9	*20	2	14	18	4	0.28	0	0	0	0	0	0	0	9
12652	7	*20	0.51	1.92	0.67	2.26	0.07	0.03	<10	0	0	0	0	0	Ŋ
12653	2	*20	2.23	4.2	2	1.78	0.21	<10	0	0	0	0	0	0	9
12643	9	20*	18.1	16.9	35.4	32.8	27.7	25.8	19.9	15.43	15.7	26.1	37.5	32.3	0
12649	9	20*	9.2	18.4	22	24.6	40	15.7	12.6	18.4	15	29.6	8.9	16.9	•
12667	9	20*	3.8	9	16.9	30	8.1	4.9	5.9	6	6.9	12.3	1.8	7.6	0
12744	7	10**	1.1	0.22	<10	<10	0	0	0	0	0	0	0	0	Ø
12754	9	10**	3.8	12.8	35	17.9	20	0.91	0.12	0.11	<10	0	0	0	m
12755	9	10**	2.1	9.2	40.1	21.5	24.9	3.5	1.5	0.36	<10	0	0	0	ę
12659 Control	_		6.1	20	21	23	5.6	-	0.82	0.22	<10	<10	0	0	7
*= Prochlorper; **= Chloroquin	azine														

TABLE 2

SUMMARY OF ACTIVITY OF WR280003AC (BN 43106) PROCHLORPERAZINE AND WR 1544 BM (AR 20613) CHLOROQUINE ALONE OR IN COMBINATION AGAINST INFECTIONS OF THE AMRU-1 STRAIN (COR) OF *Plasmodium vivax* IN *AOTUS*

nkey No.	Daily Dose x 7	L Z	Response of Parasitemia, Summer	to RX	Days from initial Rx to parasite	Days from Final Rx to Recrudes-	Notes No of days negative	
	6v/6ini		ouppiessed	Created	Clearance	cence		
643		Ŧ			37	n.a.	61	
649	*20	+		s_	19	•	1, Died/anemia	
667	*20	+		×	18	4	74	
651	*20			÷	4	n.a.	68	
	* * 10							22
352	*20			÷	7	n.a	85	!
	* * 10							
653	*20			+	വ	n.a.	87	
	* *10							
744	* * 10			+	ო	n.a.	88	
754	* * 10			+	ω	n.a.	8, Died/anemia	
755	* * 10			+	ω	n.a.	84	

* Prochlorperazine ** Chloroquine

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DETAILED PARASITEMIA OF A0TUS MONKEYS VACCINATED WITH A PLASMID DNA AMA-1 VACCINE AND CHALLENGED WITH AN FVO STRAIN OF PLASMODIUM FALCIPARUM

PI/DAY = Post inoculation day * = day of initiation of treatment with mefloquine Parasitemia = parasites x ml of blood

		DET, V/	AILED P ACCINE	ARASITI AND RE	EMIA OF	<i>: AOTUS</i> ENGED V	MONKEY VITH AN	S VACC FVO STF	SINATED SAIN OF	WITH A PLASMO	PLASM PDIUM I	IID DNA FALCIPA	AMA-1 RUM	
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12/8/ 12789	0 v 10	× 10 • 10	<pre>< 10</pre>	v v 10 10	< 10< 10<	< 10< 10<	<pre>< 10</pre>	<pre>> 10 > 10 > 10</pre>	ر 10 ×	0 <10	v v 10 10	× 10 × 10	1010	<pre>< 10</pre>
12790	0	< 10	0	0	0	0	0	0	< 10	< 10	< 10	12910	24660	9240
12791	0	< 10	0	0	0	0	0 ⁴	0	< 10	< 10	< 10	3620	16940	30800
12792	< 10	< 10	< 10	< 10	910	810	<10	<10	< 10	<10	< 10	< 10	< 10	0
12793	<10	< 10	> 10	< 10	1420	16940	3000	370	530	12320	2180	30800	22710	86240
PI/DAY	21	22	23	24	25	26	27	28	29					
12770	260	* < 10												
12787	< 10	> 10	< 10	< 10	< 10	< 10	< 10	< 10	* < 10					
12789	< 10	< 10	<10	< 10	<10	0	0	0	0					
12790	94800	16940	7840	*1580										
12791	9100	27720	10780	*2050										
12793	0 4280	2 0 2 0	< 10	* < 10										
	PI/DAΥ = * = day c	= Post ino of initiation	culation (n of treat	day ment with	mefloquir	ЭС								
	Parasiter	mia = para	asites x rr	l of blood										

TABLE 4

-24-

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TABLE 6

CHALLENGE WITH THE FVO STRAIN OF *PLASMODIUM FALCIPARUM*

MONK	NO. OF	NOTES
<u>NO.</u>	CHALLENGES	
10707	G	
12727	6	Sterile immunity
12730	6	Sterile immunity
12735	6	Sterile immunity
12739	6	Sterile immunity
12762	5	Sterile immunity
12749	5	Sterile immunity
12748	4	Sterile immunity
12756	4	Sterile immunity
12757	4	Sterile immunity
12759	4	Sterile immunity
12763	4	Sterile immunity
12765	4	Sterile immunity
12752	4	Not immune/Died/49 days/Pl
12764	3	Died Malaria/25 days/Pl
12169	2	Died day 32 days/PI, malaria
12687	2	Rx, died day 46 days/PI, inter-
		current infection
12738	2	Died day 19/PI, malaria
12740	2	Rx,died 51 days/Pl
		inter-current infection
12731	. 1	Died of Malaria 17 days/Pl
12726	1	Died of Malaria 18 days/Pl
12761	1	Died of intercurrent infection
		46 days/Pl
12768	1	Died lung aspiration17
	-	davs/Pi
12786	2	Died/Malaria 23 days/Pl
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