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13. ABSTRACT *(Maximum 200 words)*

Cooperative Agreement #DAMD17-95-2-5001 was implemented 15 October 1994 to provide funding support for Royal Thai Army investigators at the Armed Forces Research Institute of Medical Sciences (AFRIMS) engaged in research activities in collaboration with US Army investigators. The principal focus of research under the agreement is directed to activities to prepare for development and testing of vaccine(s) for the prevention of HIV infection and/or disease. During the reporting period, research activities were directed in 3 primary areas 1) study of the natural history of HIV infection/disease in Thais to define and establish endpoints for projected vaccine efficacy testing; 2) cohort development studies to define an appropriate population(s) for vaccine testing; and 3) implementation of phase I/II vaccine studies to determine safety and immunogenicity of potential vaccines in Thais. Other efforts under the cooperative agreement during the reporting period included 1) animal care and handling, including multiple small animal species and a primate colony, in support of other ongoing research activities at AFRIMS, exclusive of HIV research; and 2) site maintenance activities in support of research activities including glassware and utilities support.

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TABLE OF CONTENTS

I. INTRODUCTION

A. General

The Armed Forces Research Institute of Medical Sciences (AFRIMS) conducts research into infectious diseases with both military and public health relevance to both the United States and Royal Thai Governments. Studies leading to the prevention of HIV infections are of primary importance to the Royal Thai Army. In addition, malaria, dengue, hepatitis, Japanese encephalitis, scrub typhus, and infectious diarrhea are all areas in which we have major interest.

B. Preparations for HIV vaccine Testing

Infection with the Human Immunodeficiency Virus, type 1 (HIV-1), which causes the Acquired Immunodeficiency Syndrome (AIDS), is pandemic. Current estimates indicate that at least 15 million infections exist in 1994, growing to up to 100 million by the year 2000. 80% of infections exist in the developing world. The epidemic is currently exploding in South and Southeast Asia with 2 to 3 million infections estimated in 1994, most of which have occurred in the past 5 years.

Efforts to prevent infection with HIV-1 are currently limited to education and behavioral change, including the use of "safer" sex measures such as condoms and limitation of sexual activities to monogamous relationships with monogamous partners. These measures have so far proved to have limited effectiveness. Vaccines for the prevention of HIV-1 disease and transmission have been under development for several years with testing beginning in the United States in both seronegative and seropositive patients in 1989 and 1990.

In 1990, researchers in the Department of Defense (DoD), among others, recognized the emerging HIV-1 epidemic in Thailand which had first become apparent in 1989 in intravenous drug users (IDU's). An agreement was made with the Royal Thai Army Medical Component (RTAMC) at the Armed Forces Research Institute of Medical Sciences (AFRIMS) to embark on a program of preparation

for eventual field-efficacy evaluation of an appropriate vaccine candidate(s) for the prevention of HIV-1 disease and transmission.

Since 1991, The US Army Medical Component (USAMC) and the RTAMC at AFRIMS have conducted descriptive epidemiological studies of prevalent and incident infection with HIV-1 in Royal Thai Army conscripts, thereby contributing to the characterization of the best characterized HIV-1 epidemic in the world. In January 1993, AFRIMS opened a Joint Clinical Research Center (JCRC) for the conduct of Phase 1 (safety) trials of vaccine candidates in Bangkok. Since June of 1993, the HIV-1 research collaboration at AFRIMS has embarked on a program of cohort development to prepare a population for eventual participation in the efficacy evaluation of an appropriate HIV-1 vaccine candidate.

Many of the regions of the world where the pandemic is worst coincide with where American forces have been or are at risk to be deployed. HIV-1 is a sexually transmitted disease (STD) and hence would pose a risk for forces deployed to areas where HIV-1 is epidemic. This phenomenon has been readily observed with United Nations (UN) forces which have been deployed on "blue helmet" missions to countries such as Cambodia and Mozambique. Additionally, over 8,000 prevalent cases of HIV-1 infection within the US military are projected to cost over $1 billion for health care services within the DOD system by the end of this century. Hence there is a military relevance to the development of preventive measures for the prevention of HIV-1 disease and transmission, including, especially, an effective vaccine for the prevention of HIV-1 disease and transmission.

C. Studies Using Animals

Most of the diseases studied at AFRIMS, including malaria, infectious diarrhea, dengue, hepatitis, scrub typhus and Japanese encephalitis, involve the use of animals as models of human disease. Animal models have repeatedly shown to be accurate predictors of results in human vaccine, pathogenesis, toxicology and therapeutic agent studies, and thus are used extensively at AFRIMS. For example, one study has used the rhesus

monkey/primate malaria model to select the most promising candidate for a human sporozoite-based malaria vaccine from among 19 advanced formulations. These studies employed 100 monkeys, and were a well-concerted effort between the Departments of Immunology and Veterinary Medicine in the USAMC. The same departments are collaborating on another study, a request by the US food and Drug Administration (FDA), to test the toxicity of the first human malaria transmission-blocking vaccine in monkeys. This study is currently underway at AFRIMS in 16 monkeys.

Similar studies are on-going to develop vaccines, either initial or improved, against dengue, Japanese encephalitis, shigella, and hepatitis E. These studies will require the use of many more monkeys, rabbits, mice, rats and possibly other animals shown to be of value in such studies. The importance and significance of being able to properly use animals in most of infectious disease studies cannot be over-emphasized. Future use of animals at AFRIMS will not diminish, and will probably increase.

D. Laboratory Science Support

The glassware section provides glassware cleaning and support to all science departments at AFRIMS. This support is of fundamental importance to the ongoing research activities at AFRIMS and includes stocking commonly used items of glassware and the proper disinfection, cleaning, and/or sterilizing of all laboratory glassware. The glassware section is also responsible for the daily production of sufficient quantities of pure de-ionized water for use in laboratory assays which cannot otherwise be performed properly

E. Space and Utilities Required

Funding under the cooperative agreement is also directed by the Principal Investigator to the provision of site maintenance including space and utilities management for both the RTAMC and the USAMC in support of research activities.

II. BODY

A. General

Efforts made under the cooperative agreement during FY95 are focused in three general areas: 1) preparations for HIV vaccine testing; 2) animal care and handling in support of ongoing research at AFRIMS; and 3) site maintenance and laboratory support activities. In general, efforts have been successful, in spite of many scientific and non-scientific obstacles.

B. Preparations for HIV Vaccine Testing

1. Natural History Study

a. Introduction

Understanding the natural history of HIV-1 infection is essential to planning for a phase III vaccine trial. There are many possible outcomes in the vaccinated subject who subsequently becomes exposed to HIV-1. In the best case scenario, HIV-1 vaccines may prevent infection (sterilizing immunity). However, protective vaccines (e.g. live attenuated polio vaccine) are thought to provide their clinical benefit through limiting (but not preventing) virus replication after challenge. Hence, although the induction of sterilizing immunity may be the ideal outcome in an HIV vaccine study, a product which induces an immune response which modifies viral replication, disease progression, or subsequent transmission is the more likely outcome.

Conceivably, vaccinees who are subsequently exposed to HIV-1 may demonstrate a booster effect of the immune response without infection, transient abortive infection, low grade controlled infection with a low viral load, unchanged symptoms of infection and viral load or, in the worst case, infection with higher than expected viral load, more severe symptoms and accelerated disease.

Identification of the immune responses that can protect humans from either HIV-1 infection or disease has not

been made. Furthermore, there are no parallel animal models of human disease with HIV-1 to provide information on correlates of immune protection.

Valuable information about the natural history of HIV infection has come from prospective follow-up of cohorts of people at high risk of infection: homosexual males, hemophiliacs and intravenous drug users. As those in these cohorts become infected with HIV-1, the progression of the disease can be followed prospectively from the start of the infection. Because the time since infection is such an important predictor of progression, it is vital to study an incident cohort, that is, a cohort of people whose date of infection is known.

Almost all incident cohorts being studied at this time consist of males in Europe or North America, in most cases of Caucasian ancestry and infected with HIV-1, subtype B. There are many reasons to think that disease progression in the developing world might be different from that in the developed world, but there is very little data available to assess the question. Data from a prospectively followed cohort of commercial sex workers in Kenya show much more rapid progression of disease than has been reported in other cohorts. Hypotheses about the reasons for this difference are easy to generate and difficult to prove without following other seroincident cohorts in the developing world. No information is currently available about the pathogenicity of subtype E, the predominant subtype in Thailand and whether the disease progression of those infected with E is significantly different from those infected with other subtypes, especially subtype B.

b. Study objectives

(1) To characterize viral, immune regulatory and clinical sequelae in recently HIV-1 infected Thai men, during the first three years post-infection. These data may form the basis of efficacy endpoints in future prophylactic vaccine trials in Thailand;

(2) To characterize (genetically and serologically) circulating HIV-1 from recently infected Thais. These data may form the basis for selection of vaccine strain prototypes for use in development of Thai-specific vaccine constructs; and

(3) To assess virus specific and immune regulatory correlates.

c. Study methods

Study population

This protocol contains plans for study of three groups of subjects: a prospective study of a seroincident cases, a cross-sectional study of prospective cases and an evaluation of uninfected persons. The first groups will be followed in order to document the natural history of infection during the first few years after infection. The second study is a cross-sectional look at prevalent HIV-1 patients representing the full range of HIV disease in Thailand. The third group will provide data on normal values for the Thai population and serve as a control group for the other two populations studies.

Seroincident cases

Persons with incident HIV infections from cohort studies in Thailand are recruited for this study. If willing, they sign a consent form to take part in the study. At that time they donate 50 ml of blood. The subjects also receive a physical examination and a brief questionnaire requesting information about their risk behaviors and recent medical history. The seroincident subjects are asked to return every 6 months for three years.

Seroprevalent HIV-infected Thais

HIV-infected subjects who enroll in this study are referred to the AFRIMS clinic from local physicians collaborating in the study.

Thais without HIV-1 infection

Uninfected Thais in the study include Royal Thai Army recruits and personnel who work at AFRIMS.

Laboratory methods

At the time of enrollment and at follow-up visits, a complete cell count (CBC) and lymphocyte immunophenotyping is done on all subjects. PCR is also conducted on seroincident and seroprevalent cases. Cells, plasma and sera are archived from each subject for future testing. Other testing, described below, will be done on a selected basis:

CBC and lymphocyte phenotyping

CBC and differential are measured using the Coulter MaxM counter. Lymphocyte immunophenotyping is performed using dual flourescent staining and analyzed on the Facscan using Simulset software at AFRIMS.

PCR subtyping

Primary PBMC derived DNA is used for PCR typing. HIV-1 subtypes are differentiated by nested PCR using primers in the gp 41 *env* region. Second round primers differentiated clades B and E, with the amplication of a 287 bp product.

Quantitative RNA PCR

Virus is pelleted at 12,000g for 1h at $4^{o}c$, followed by lysis. RT-PCR is conducted using gag primers. Liquid hybridization with radiolabelled probes, followed by quantitative detection of hybridised produce with

phosphorimaging. The assay is sensitive to 10^2 copies RNA/ml plasma.

P24 antigen

P24 Ag is quantitatively assessed in serum using an immune-complex dissociation assay (Coulter).

d. Results

Study enrollment

Incident cases	91
Prevalent case	275
Seronegative case	99

The results presented (Tables 1 -3) here include incident cases enrolled in the natural history study and results from subjects who are being followed under different protocols which allow follow-up of seroincident cases, but whose blood are being tested at AFRIMS. Table 1 shows the basic characteristics of all three groups of subjects. Table 2 shows more detailed information on the characteristics of the incident cases.

Table 1 - Study population demographics

	Incident Cases* n=91		Uninfected n=99		Prevalent Cases+ n= 275	
	n	(%)	n	(%)	n	(%)
Age						
<20	2	(2)	0	(0)	0	(0)
20-29	81	(82)	64	(70)	176	(64)
30-39	5	(5)	20	(22)	86	(31)
40-49	-	-	-	-	12	(4)
Unk	3	(3)	5	(7)	1	(0)
Sex						
Male	79	(87)	75	(76)	180	(65)
Female	12	(13)	24	(26)	95	(35)
Home Region						
Central	3	(4)	10	(10)	54	(20)
Northeast	4	(4)	42	(43)	21	(8)
North	65	(71)	11	(11)	42	(15)
Bangkok	12	(13)	32	(32)	135	(49)
South	-	-	-	-	1	(0)
Unk	7	(8)	3	(4)	20	(4)

* includes subjects tested, who were enrolled in other prospective studies
+ does not include prevalent cases for whom CD4 counts have been provided as a 'service' to the Phramongkutklao Hospital HIV clinic

Table 2 - Summary of incident cases (n=91)

	n	(%)
Subtype (n=74)		
E	68	(92)
B	1	(2)
UN	5	(6)
Cohort enrolled from		
CSW	11	(13)
RTA recruits	69	(79)
STD clinic	9	(7)
Other	2	(2)
Estimated month of seroconversion		
Before July 1993	69	(76)
July 93-Dec 93	11	(14)
Jan 94-June94	7	(8)
July 94-Dec 94	1	(1)
Jan 95-June 95	1	(1)
IDU Use (n=77)		
Yes	2	(3)
No	75	(97)
Estimated time since seroconversion		
<12 months	54	(60)
12-23 months	35	(39)
>24 months	2	(1)
Number of follow-up visits[+]		
1	59	(65)
2	19	(21)
3	7	(8)
4	6	(7)

+ of the incident cases, only 28 are still in follow-up

Table 3 - Characteristics of incident cases with more than one blood draw (n=30)

	n	*(%)*
Sex		
male	26	(87)
female	4	(13)
Time from seroconversion to first blood		
<6 mos	8	(27)
6-11 mos	12	(40)
12-23 mos	10	(33)
Time from seroconversion to last blood draw		
<12 mos	2	(6)
12-23 mos	20	(67)
24-35 mos	8	(27)
Time between first and last blood draw		
<6 mos	4	(13)
6-11 mos	14	(47)
12-23 mos	12	(40)
Change in CD4 count between first and last blood draws		
increase	11	(37)
decrease	19	(63)

Evaluation of CD4 counts in incident cases and in uninfected persons

A preliminary analysis of incident cases and seronegative cases has been undertaken to evaluate disease progression as determined by CD4 counts. A small percentage of incident cases have returned for more than one visit, and a cross-sectional determination of CD4 counts by time since estimated seroconversion is available. These data were presented as an abstract and suggest that CD4 counts and percents are lower both in HIV infected and in HIV uninfected Thais than in US populations.

P24 antigen

ICD P24 antigen was detected in 50% (33/67) of persons with HIV infection. There were no differences in the percent of persons positive either by time since seroconversion (<6 vs 6-11 vs 12-23 months) or by CD4 count (<400 vs >=400).

RNA viral load

Viral load was determined on a subset of incident cases to determine the mean viral load in this population.

Plasma specimens, collected in heparin and EDTA, from prevalent cases have been tested to compare the WRAIR and Roche viral load assays for subtype E. This comparison indicated that caution should be used when using the Roche assay to assess viral burden on HIV specimens which are not of the B clade.

Dual infection

Specimens collected under this protocol were used for positive identification of two individuals who have been infected with two different subtypes of HIV-1. This work has been completed and published.

Lymphoproliferation

Lymphoproliferation assays to standard mitogens and common microbial recall antigens are being evaluated. Responsiveness to candida and PPD has been found to be inversely correlated to CD4 count. Efforts to measure responsiveness to HIV-1 antigens has been hampered by the lack of purified antigens, although there is some suggestion of envelope specific responses in HIV-1 infected patients with CD4 counts>400/cu mm (attachment 4).

Assay development

FACSCount

A comparison of CD4, CD8, and CD3 absolute counts between the Becton-Dickinson FACSCount™ and FACScan™ has been conducted under this protocol using blood collected from prevalent and incident cases. A total of 77 cases has been studied. These data indicate that the FACSCount™ is an acceptable alternative to the FACScan™ for CD4 counts (R=0.944). A similar correlation was found for CD8 and CD3 cell counts.

Comparision of subtype determinations

A panel of Thai specimens is being used in a three-way validation project incorporating an HIV serologic assay, a nested PCR assay which differentiates HIV subtype B from subtype E (FM primers), and heteroduplex mobility assay (HMA). Over 30 specimens have shown 100% concordance between HMA and FM primer typing.

Characterization of in vitro growth kinetics of HIV subtype E

The *in vitro* growth characteristics of primary isolates of HIV subtype E have not been extensively studied. A project is underway to assess the growth of HIV subtype E in PBMC, with further characterization in monocytes and lymphocytes derived from PBMC. Factors such as the method of isolation (standard cocultivation, plasma or directly from PBMC) in relation to subsequent passages of the virus will be monitored, as well as the phenotype of

the virus and the source of cultured virus on further infection.

Plasma viral RNA quantitation

A plasma HIV RNA quantitative assay is being developed for use on incident cases of HIV infection. The assay uses a reverse transcriptase step employing a *pol* oligonucleotide, followed by amplification of the cDNA using PCR (*pol* primers), and product visualisation using ethidium bromide. Dilutions of a reference plasmid of known copy number serve as the positive control and are used to construct the standard curve. The detection method is currently insensitive, with a minimal detection limit of 10^4 copies/ml. Liquid hybridization using ^{32}P and detection with a phosphor imager are under consideration.

2. Cohort Studies

Cohort development for Phase III trials is ongoing. Cohort development includes planning recruitment and follow-up mechanisms and determination of follow-up rates, HIV-1 incidence, behavior and STD rates in the population. Data collected from routine HIV-1 surveillance being conducted in the RTA, as well as several HIV-1 cohort studies, will provide information concerning cohorts which might be suitable for Phase III trials. Because the HIV epidemic in Thailand is dynamic and there are rapid changes occurring in the society, the process of identifying a suitable cohort has been challenging. Feasibility studies in two cohorts were begun in FY95.

Prevalence and incidence of HIV-1 infections among recruits in the Royal Thai Army at Prachuab Khiri Khan

a. Introduction

Numerous studies have focused on the incidence and prevalence of HIV-1 infection among Royal Thai Army conscripts (Tahan Gan). RTA conscript populations are socio-demographically homogeneous as relatively advantaged populations are excluded from conscription. Conscripts tend to be from non-municipal areas, engaged in agrarian occupations, possess a primary school education, and come from a Buddhist background. Those studies examining risk factors, interventions, or follow-up have focused on recruits in the Northern region where the epidemic has been most prominent.

Prachuap Khiri Khan is the southernmost province of the Central region. Although the Central and Southern regions have not been as highly associated with the HIV epidemic in Thailand, prevalence data from the Royal Thai Army on recruits assigned to Prachuap Khiri Khan have shown a steady increase. This is in part due to a number of recruits assigned to this province from other regions, but there has also been a significant increase in the prevalence in recruits from the Central and Southern regions (as well as from the Northeast). Epidemiological and social/behavioral research has indicated an absence

of major differences in patterns of risk behaviors present between these regions. Prachuap Khiri Khan was selected from other provinces as it had a large recruit population, increasing prevalence as mentioned above, predominantly non deploying units simplifying follow-up, and a single large hospital responsible for care. The start date for this study was July 1995.

b. Study objectives

(1) Study the prevalence and incidence of HIV-1 infection in recruits stationed at two army bases in Prachuap Khiri Khan province in the Central region of Thailand.

(2) Study the attitudes, behavior and follow-up patterns in the recruits.

c. Methods

HIV-1 testing is being done at baseline and every 6 months. At each bleed, a questionnaire is administered to evaluate behavior and knowledge. Two different educational and behavioral intervention programs are being implemented. The incidence of HIV-1 in the recruits, over all and in the two intervention groups, will be determined. At the end of the followup period, subjects will complete a questionnaire to assess attitudes towards participation in vaccine trials.

d. Results

Number of subjects enrolled: 967

Number of subjects to be enrolled: up to 5,000

Enrollment was considerably more complicated than originally expected. Although these conscripts are assigned to units located at the two bases, many recruits actually serve with small detachments of these units located at distances from the bases. Information is currently being collected on obstacles to enrollment and follow-up.

Incidence of HIV-1 infection among persons attending STD clinics and anonymous test sites

a. Introduction

This protocol studies the prevalence and incidence of HIV-1 infection in persons attending STD clinics in several areas of Thailand to determine whether this group would be a feasible cohort for HIV vaccine efficacy trials. The start date for the study was Sept 1995.

b. Study objectives

(1) Study the prevalence and incidence of HIV-1 infection in persons attending STD clinics and anonymous test sites.

(2) Study the attitudes, behavior and follow-up patterns in the cohort.

c. Methods

Subjects are enrolled from STD clinics and anonymous test sites at three sites, Bangkok, Chonburi, and Lampang. Participants are tested for HIV-1 at 4 month intervals for one year. Education and counseling are provided at each visit. At each bleed, a questionnaire is administered to evaluate behavior and knowledge. At the end of the follow-up period, subjects will complete a questionnaire to assess attitudes towards participation in vaccine trials.

d. Results

Subjects enrolled: 120

Subject to be enrolled: 500-1000

3. *HIV-1 Vaccine Testing*

Screening and evaluation of potential volunteers

a. Introduction

In order to evaluate Thai volunteers for suitability for participation in clinical safety and immunogenicity trials (Phase I and II) of candidate vaccines for the prevention of HIV-1, a screening protocol was developed. Information gathered from these volunteers will be used to refine recruitment, screening and inclusion criteria for current and future vaccine studies.

Recruitment and screening of volunteers for HIV vaccine trials is necessary for the success of vaccine trials; however, the techniques and methods for successful recruitment for HIV vaccine trials are unproved and virtually untried in Thailand. Volunteers for all vaccine trials will be required to have clinical and laboratory characteristics which will be generally constant for all trials. Therefore, screening for potential vaccine trial subjects can be independent of the particulars anticipated vaccine trials. The ability to begin screening volunteers under a human use approved protocol, according to criteria which satisfy inclusion and exclusion criteria for the actual vaccine trial 30 to 60 days in advance of actual trial approval should allow a more rapid implementation and enrollment phase for each vaccine trial.

Information from this protocol may guide future recruitment strategies. Additionally, characterization of those who are screened under this protocol but do not qualify for enrollment into vaccine trials may be useful in the design of future HIV research protocols in Thailand.

b. Methods

The study is conducted at two sites: the Joint Clinical Research Center (JCRC) at AFRIMS in Bangkok and the RIHES Vaccine Trial Unit at the Research Institute of

Health Science (RIHES) at Chiang Mai University in Chiang Mai.

Recruitment activities began in late CY1994 and extended through August 1995 in both Chiang Mai and Bangkok. Activities included information booths at community fairs and other community meetings; presentations to medical professionals and para-professionals; radio and television ads and interviews; newspaper ads; and distribution of posters and brochures to local businesses, AIDS-related NGO's and retail stores. Ads and posters which were distributed had received prior approval for distribution by the HURRAD.

Evaluation of volunteers includes collection of demographic information, medical history, laboratory evaluation (including CBC, lymphocyte immunophenotyping, serum ALT and creatinine, CXR, HBsAg, pregnancy test,and RPR) and in depth psychological and HIV-risk assessment.

c. Results

Bangkok: Over 200 telephone and personal inquiries resulted from the recruitment efforts. The first volunteer was enrolled 1 August 1995. As of October 19, 1995,52 volunteers were enrolled to find 21 candidates suitable for enrollment in the first vaccine trial.

Chiang Mai: Over 400 potential volunteers have been contacted as a result of recruiting efforts. The first volunteer was enrolled 1 August 1995. As of October 19, 1995, 47 volunteers were enrolled for screening to find 26 candidates suitable for enrollment in the first vaccine trial.

Table 4 - Characteristics of volunteers in vaccine screening protocol (through 20 October 1995)

	BANGKOK (n=52)	CHIANG MAI (n=47)
mean age:	30 years (median=29)	33 years (median=32)
male:female	40:12 (77% male)	31:16 (66% male)
marital	35 single (67%)	19 single (40%)
status	9 married (17%)	21 married (45%)
	6 divorced (12%)	3 divorced (6%)
	2 widowed (4%)	4 widowed (8%)
education:	1 Medical Degree (2%)	2 Master's Degree (4%)
	2 Master's Degree (4%)	10 Bachelor's degree (21%)
	21 Bachelor's Degree (40%)	5 vocational school (10%)
	9 vocational school (17%)	9 senior high school 19%)
	10 senior high school(19%)	7 junior high school (15%)
	2 junior high school (4%)	13 primary school (28%)
	7 primary school (13%)	1 not recorded
occupation	8 business owners	5 administrative
	5 engineers	5 monks
	4 state ent/gov't officers	5 students
	3 state ent/gov't employees	4 caddies
	3 office clerks	4 laborers
	3 unemployed	2 health educators
	3 university students	2 housekeepers
	2 sales representatives	2 health workers
	2 monks	2 electricians
	1 physician	2 forest rangers
	1 medical student	2 teacher
	1 security guard	1 chef
	1 marketing supervisor	1 driver
	1 driver	1 farmer
	2 taxi driver (1 motorcycle)	1 cleaner
	1 construction supervisor	1 carpenter
	1 factory worker	1 community worker
	1 military officer	1 beautician
	1 banker	1 reporter
	3 allied health	1 nurse
	1 teacher	1 nutritionist
	1 hospital receptionist	1 plumber
	1 telephone operator	1 soldier
	1 hotel manager	

Phase I trial of Biocine HIV SF2 gp120/MF59 vaccine

a. Introduction

This double-blind, randomized, Phase I study evaluates the safety/tolerability and immunogenicity of the BIOCINE Human Immunodeficiency Virus (HIV) SF2 gp120/MF59 Vaccine at the dose of 50μg in two immunization schedules.

b. Study objectives

(1) Are there any acute adverse clinical reactions or laboratory evidence of toxicity to the candidate vaccine (i.e., local or systemic reactions or immunologic impairment)?

(2) Does the vaccine induce anti-HIV- I antibodies? In particular, does it stimulate anti-HIV neutralizing antibody to, at least, the homologous viral subtype (clade) represented in this product when administered to healthy volunteers?

(3) Is the safety, tolerability and immunogenicity profile comparable with two different immunization schedules?

(4) Does the candidate vaccine stimulate the production of a lymphoproliferative response to HIV- I as represented by antigens present in this vaccine candidate and other HIV- I envelope antigens?

(5) Is the safety and immunogenicity profile of this vaccine similar in Thai and U.S. citizens?

c. Study population

The study population consists of fifty-two (52), HIV-1 seronegative, healthy Thai adults enrolled from the community at two sites, twenty-six at (AFRIMS), Bangkok, and twenty-six at in Chiang Mai.

d. Methods

After receiving ethical and scientific approval from all relevant institutional review boards (IRB's) and from the

Ethics committee of the Royal Thai Ministry of Public Health and the Scientific Subcommittee of the Thai National AIDS Prevention and Control Committee, the trial was initiated on 29 August 1995.

Subjects were enrolled after being screened in the protocol established for screening and evaluation of potential volunteers (see section B.3 above).

Subjects are randomized to receive vaccine (n=20 each of Group A1 and B1) or placebo (n=6 in each of Group A2 and B2). Two immunization schedules will be compared. Volunteers enrolled in Group A1 and A2 are vaccinated at 0, 1, and 4 months; volunteers enrolled in Groups B1 and B2 are vaccinated at 0, 1, and 6 months. Subjects are followed for four months after the third immunization. In addition, all subjects will be contacted annually for a period of up to five years to monitor their general health after the last immunization according to guidelines outlined in the National Plan for AIDS Vaccine Development and Evaluation.

All subjects and the clinic personnel are blinded to the treatment arm into which subjects are enrolled. Subjects are observed for 30 minutes following immunization for evidence of immediate local and systemic reactions. They will be instructed to watch for local (i.e., pain, fatigue, headache, nausea, myalgia, arthralgia, etc.) for seven days post-immunization. They will be contacted by the investigators within 24 to 72 hours post-immunization by telephone or by home visit to assess any symptoms reported. Evaluation by the investigator is scheduled if significant symptoms are reported. All adverse events are monitored until resolution. Vaccine immunogenicity is assessed by gp120 Enzyme-Linked Immunosorbent Assay (ELISA) and HIV neutralizing antibody assays, as well as by specific lymphocyte proliferation. Immunogenicity of the two injection schedules will be compared.

A preliminary safety analysis will be performed after the second immunization. A safety and immunogenicity interim

analysis will be performed after the third immunization. A final analysis will occur after all subject visits are complete. The goal of this Phase I trial is to be the first of a set of studies that ultimately should evaluate formulations consisting of Clade B and E antigens.

e. Results

Subject Enrollment

Number of subjects enrolled (31 October 1995)		47
Bangkok	21	
Chiang Mai	26	
Target enrollment		52

The characteristics of the subjects are presented in table 5.

Table 5 - Characteristics of subjects enrolled in Phase I study of Biocine® HIV SF2 gp160/MF59 vaccine (through 19 October 1995)

	BANGKOK	CHIANG MAI
# enrolled	21 (41%)	25 (53%)
enrolled by exception	8 (38%)	8 (32%)
mean age	30 years (median=29)	34 years (median=34)
male:female	15:6 (71% male)	16:9 (64% male)
marital status	16 single (76%) 1 married (5%) 3 divorced (14%) 1 widowed (5%)	10 single (40%) 11 married (44%) 2 divorced (8%) 2 widowed (8%)
education	1 Medical Degree (5%) 1 Master's Degree (5%) 8 Bachelor's Degree (38%) 4 vocational school (19%) 2 sr high school (10%) 1 jr high school (5%) 3 primary school (14%)	1 Master's Degree (4%) 7 Bachelor's Degree (28%) 2 vocational school (8%) 5 sr high school (20%) 3 jr high school (12%) 6 primary school (24%) 1 not recorded
occupation	4 business owners 2 state enterprise officers 2 university students 2 sales representatives (one sells traditional medicines) 1 physician 1 marketing supervisor 1 driver 1 taxi driver 1 government employee 1 monk 1 construction supervisor 1 factory worker 1 engineer 1 military officer 1 banker	5 administrative 3 monks 2 students 2 laborers 2 health workers 1 driver 1 farmer 1 cleaner 1 carpenter 1 nurse 1 community worker 1 forest ranger 1 beautician 1 housekeeper 1 reporter 1 teacher

Reactogenicity

There have been no serious or unexpected adverse events in the first 47 subjects who have received at least one dose of vaccine.

4. *Surveillance*

a. Introduction

A previous nationwide seroprevalence survey with demographic data collection was conducted on Royal Thai Army conscripts from November 1991 to May 1993. This survey allowed definition of the epidemic nationwide and has assisted both the Ministry of Defense, the Ministry of Public Health, and other Royal Thai Government agencies to better understand the epidemic in Thailand.

This project studies the prevalence nationwide among recruits serving with the Royal Thai Army in Thailand and will assess temporal, geographic and demographic correlates of HIV-1 infection among the young men. The information obtained from this study will help monitor the epidemic and assist in identification of location for potential cohorts for Phase III trials.

b. Methods

Demographic information is collected on young men entering service with the Royal Thai Army (RTA) nationwide and is merged with routine serologic HIV data collected by the RTA. The recruits are bled at entry into the RTA (every November and May). Sera are testing for HIV by ELISA and positives are confirmed by Western Blot.

Data from this study will be analyzed, along with data from RV70 (a previous project which had a similar design) to evaluate trends.

c. Results

Enrollment: 60,000 recruits

Subjects to be enrolled: 180,000-210,000

Trends in seroprevalence in the RTA

Compared with 1992-1993, the prevalence of HIV-1 infection in RTA recruits has decreased nationwide. The decrease was observed in all regions and at all educational levels.

Table 6 - HIV-1 seroprevalence by region of service, 1992 to 1994

Region of Service	1992 Positive/Total (%)	1993 Positive/Total (%)	1994 Positive/Total (%)
Northeast	297/12557 (2.4)	260/10139 (2.6)	292/11301 (2.6)
South	141/5314 (2.7)	135/4833 (2.8)	103/4740 (2.2)
Bangkok	278/8323 (3.3)	265/8199 (3.2)	241/8301 (2.9)
Central	688/24049 (2.9)	607/20158 (3.0)	571/22344 (2.6)
North	699/9331 (7.5)	649/8889 (7.3)	441/8662 (5.1)*
Upper North	440/3549 (12.4)	384/3436 (11.2)	278/3525 (7.9)*
Lower North	259/5782 (4.5)	265/5453 (4.9)	163/5137 (3.2)*
Total	2103/59474 (3.5)	1916/52218 (3.7)	1648/55348 (3.0)*

*$p<0.001$ χ^2 test for trend 1992-1994

Table 7 - HIV-1 seroprevalence and percent change by demographic factors for November 1992 and November 1994

	November 1992 Positive/Total (%)		November 1994 Positive/Total (%)		Percent Change
Number of recruits tested	1001/28787	(3.5)	693/26513	(2.6)	-25*
Age (years)					
21	936/27195	(3.4)	616/25063	(2.5)	-29*
22-29	55/1328	(4.1)	48/960	(5.0)	21
Level of education (years)					
0-6	758/22067	(3.4)	519/19971	(2.6)	-24*
7-9	158/3924	(4.0)	96/3379	(2.8)	-29†
10-12	62/2045	(3.0)	46/2089	(2.2)	-27
13-16	6/384	(1.6)	3/527	(0.6)	-64
Area of residence in previous 2 years					
Rural	571/17579	(3.2)	343/14048	(2.4)	-25*
Municipal	284/6782	(4.2)	204/7301	(2.8)	-33*
Region of residence in previous 2 years					
Northeast	354/16365	(2.2)	279/14490	(1.9)	-11
South	42/1507	(2.8)	27/1136	(2.4)	-15
Bangkok	90/2557	(3.5)	66/2906	(2.3)	-36†
Central	133/2452	(5.4)	106/2871	(3.7)	-32†
North	359/5173	(6.9)	168/4037	(4.2)	-40*
Upper north	273/2042	(13.4)	121/1722	(7.0)	-47*
Lower north	86/3131	(2.7)	47/2315	(2.0)	-26
Region of service					
Northeast	152/6169	(2.5)	140/5395	(2.6)	5
South	96/2368	(4.1)	63/2081	(3.0)	-25
Bangkok	106/3985	(2.7)	76/4065	(1.9)	-30‡
Central	295/11820	(2.5)	213/10965	(1.9)	-22†
North	352/4445	(7.9)	201/4007	(5.0)	-37*
Upper north	222/1710	(13.0)	130/1667	(7.8)	-40*
Lower north	130/2735	(4.8)	71/2340	(3.0)	-36†

*p<0.0001

†p<0.01

‡p<0.05

Evaluation of RIBA

An evaluation of the RIBA was conducted using sera from a random sample of ELISA negative and a panel of ELISA negative/Western Blot (WB) indeterminant or negative sera from the RTA conscripts. No positive or indeterminate RIBA results were observed on testing of ELISA negative sera (n=167). The RIBA successfully resolved most ELISA negative/WB indeterminate or negative sera.

B. Studies Using Animals

a. Introduction

The Department of Veterinary Medicine provides support for multiple animal-based research efforts. To meet the needs of researchers, the Department breeds, maintains and employs a sufficient number of animals to support an average of twenty active animal-based protocols on an ongoing basis. Over 5,000 animals of 10 different species, including three non-human primate species and four rodent species. A total of approximately 50,000 animals are utilized annually.

b. Results

During FY95 care and handling were provided for 60,688 animals in support of 17 research studies as shown in the table.

Table 8 - Animal utilization, AFRIMS, FY95

Animal Species	Number of Animals	Number of studies*
Rhesus monkey	121	8
Mice	54,456	9
Hamster	442	2
R. rattus rat	198	1
Guinea Pig	76	1
R. rattus rat,	10	QA
B. savilei rat	7	QA
Guinea pig	12	QA
All species	5,257	Breeding colonies

*Some studies utilized multiple species, therefore the total in this column is > 17.

C. Laboratory Science Support

a. Introduction

The ready availability of proper cleaning and decontamination of laboratory glassware is a fundamental

requirement for all science departments at AFRIMS. The glassware section currently supports 28 separate categories of glassware stock and stocks over 13,000 glassware items on a continuing basis.

b. Results

During FY95, the glassware section received 43,215 items of glassware for cleaning and decontamination. They distributed 37,260 items for use by various departments at AFRIMS.

Table 9 - Average daily glassware use, AFRIMS, FY95

Department	Flasks	Bottles	Beakers	Cylinders	Tubes	Pipets
Virology	50	100	50	20	400	100
Immunology	30	50	20	10	200	
Entomology	20	30	15	10		
Medicine	10	20	20	10		
Bacteriology	120	50	20	15	500	50
Retrovirology	10	15	20	5		

III. CONCLUSIONS

A. Preparations for HIV Vaccine Testing

1. Natural History Study

Due to inherent design deficiencies and unfortunate timing, the natural history study has, to date, yielded few data, of relatively limited consequence or applicability and of limited use in understanding the true natural history of HIV disease in Thailand. The natural history study has been most useful as a tool for providing reagents for laboratory strengthening and development. It has also yielded potentially useful insights for further research. A revised natural history protocol is in preparation to better address the needs of long-term followup for describing the natural history of HIV disease and defining endpoints for vaccine efficacy testing and to provide a mechanism for adequately following and evaluating vaccine subjects who develop HIV infection during vaccine trials.

2. Cohort Studies

Two cohort feasibility projects have been implemented after 18 months of preparation. The intensity of effort and resources which such undertakings demand has only become apparent with experience. At the onset of serious cohort development efforts we had planned conducting feasibility studies among 3 or 4 cohorts. It has become apparent, in retrospect, that we are ill-equipped to initiate that number of cohorts in such a short period of time.

The single-most important ingredient in successful cohort projects is a solid base of support and trust within the Thai Ministry of Public Health (MOPH) and hence with the network of ministry sponsored hospitals and clinics. In the case of the RTA, it is with hospital and base commanders that we must establish working relationships. This has been relatively simple with the RTA. However, cohorts being developed within Ministry of Public Health facilities and with civilian subjects, have required considerable efforts to establish working relationships with key individuals, including the Deputy Minister of Health, the Director of the Division of

AIDS and with numerous ministry officials at province, district and community levels.

Plans for FY96 for cohort development may include at least one additional feasibility study in a community cohort(s). Implementation of such an effort will depend upon completion of community assessments begun in FY95. Additional cohorts for possible feasibility studies will continue to be explored, recognizing the significant possibility that neither of the two (or three) cohorts currently under study may prove to be viable for vaccine testing.

3. *Phase I/II Vaccine Trial*

a. Subject Recruiting

Recruitment for this first trial was surprisingly easy, at both sites, especially given the relatively high degree of uncertainty which existed prior to the initiation of the study.

Most enrolled subjects heard about the trial from the radio or newspaper. The best response to advertising came from an article written by a locally prominent columnist in a popular daily newspaper. A smaller number responded to the distribution of posters and brochures.

b. Laboratory Screening

The relative lack of data establishing reference normal values for basic clinical laboratory assays complicated the process of screening volunteers successfully. Specifically, normal values for complete blood counts (CBC), including all measured parameters, are not well established. A significant effort will be undertaken to establish reference normals and better laboratory quality control standards for the current trial and especially for subsequent trials where product licensing requirements will be a more significant issue.

4. Surveillance

Active surveillance of RTA conscripts will continue. The data collected in this effort continues to provide one of the best windows to the dynamics of the HIV epidemic in Thailand. Future efforts are being planned to conduct serotyping, and possibly genotyping, of viral isolates to better define the virological dynamics of the epidemic, especially as regards the intrusion of new viral subtypes (e.g., subtype C) and shifting dynamics of the current subtypes, B and E.

B. Studies Using Animals

Animal-based research will continue to place a fundamental demand on Veterinary Medicine resources at AFRIMS. With expanding regulatory requirements; increasing sensitivity to animal-care issues; and a relatively constant level of ongoing or new animal-based studies, demands for a high level of animal care and handling will continue unabated and very likely increase in coming years.

C. Laboratory Science Support

The level of active research protocols, ongoing and projected will continue at historical levels or greater and will continue to require an active glassware section to meet the needs of highly technical and resource intensive scientific investigation.

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