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Cooperative Agreement # DAMD17-95-2-5001 was implemented 15 October 1994 to provide funding support for Royal Thai Army investigators at 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 prevention of HIV infection and/or disease. During the report period, research activities were directed in 4 primary areas 1) continuing and expanding studies of natural history of HIV infection/disease in Thais to define and establish endpoints for projected vaccine efficacy testing; 2) cohort development studies attempting to define an appropriate population(s) for vaccine testing; 3)conduction of phase I/II vaccine studies to determine safety and immunogenicity of potential HIV vaccines in Thais; and 4) Active surveillance of RTA conscripts to determine the dynamics of HIV epidemic in Thailand. Other efforts under the Cooperative Agreement during the reporting period included 1) animal care and handling, including multiple small animal species and 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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Gobchoke Puavilai 3rd Nov 98

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TABLE OF CONTENTS

FRONT COVER 1
SF 298 - REPORT DOCUMENTATION PAGE 2
FOREWORD 3
TABLE OF CONTENTS 4
I. INTRODUCTION 7
A. General 7
B. Preparations for HIV Vaccine Efficacy Testing 7
C. Studies Using Animals 8
D. Laboratory Science Support 8
E. Space and Utilities Required 9
II. BODY 10
A. General 10
B. Preparations for HIV Vaccine Efficacy Testing 10
1a. Natural History Study 10
a. Introduction 10
b. Study objectives 11
c. Study methods 11
d. Results 12
1b. Health Evaluation of HIV-infected and Uninfected Thai Men after Discharge from the Royal Thai Army 16
a. Introduction 16
b. Study objectives 16
c. Methods 16
d. Results 18
1c. Evaluation of Mucosal Virology and Immunity of HIV-1 in Thailand 18
a. Introduction 18
b. Study objectives 18
c. Methods 19
d. Results 19

2. Cohort Studies **19**

a. Prevalence and incidence of HIV-1 infections among recruits in the Royal Thai Army at Prachuab Khiri Khan 20

1. Introduction 20
2. Study objectives 20
3. Methods 20
4. Results 21

b. Incidence of HIV-1 infection among persons attending STD clinics and anonymous test sites 21

1. Introduction 21
2. Study objectives 21
3. Methods 21
4. Results 21

c. Incidence of HIV-1 Infection Among Women Attending Family Planning Clinics in Rayong Province, Thailand 22

1. Introduction 22
2. Studies objective 22
3. Methods 22
4. Results 24

d. Community-Based Cohort Study of HIV-1 Incidence in Sattahip, Chonburi, Thailand 24

1. Introduction 24
2. Studies objective 24
3. Methods 25
4. Results 25

3. HIV-1 Vaccine Testing **25**

a. Screening and evaluation of potential volunteers 25

1. Introduction 25
2. Methods 26
3. Results 26

b. Phase I trial of Biocine HIV SF2 gpl2O/MF59 vaccine 27

c. A Phase I/II Double -blind, Placebo Controlled of Chiron HIV Thai E gp120/MF59 Vaccine Administers Alone or Combined with the Chiron HIV Sf2 gp120/MF59 Vaccine in Healthy HIV-seronegative Thai Adults 27

1. Introduction 27
2. Study objectives 29
3. Methods 29
4. Results 31

4. Surveillance 32
a. Introduction 32
b. Methods 32
c. Results 32
C. Studies Using Animals 34
1. Introduction 34
2. Results 34
D. Laboratory Science Support 34
1. Introduction 34
2. Results 35
III. CONCLUSIONS 36
A. Preparations for HIV Vaccine Efficacy Testing 36
1. Natural History Study 36
2. Cohort Studies 36
3. Phase I/II Vaccine Trial 36
4. Surveillance 37
B. Studies Using Animals 37
C. Laboratory Science Support 37
IV. REFERENCES 38
A. Natural History Study 38
B. HIV-1 Vaccine Testing 39
C. HIV Surveillance in Thailand 39
D. Abstracts and Publications (FY1998) 41
V. PERSONNEL ASSIGNED UNDER CURRENT AGREEMENT 43

1. 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 the RTA have major interest

B. Preparations for HIV Vaccine Efficacy 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 30.6 million people were infected as of the end of 1997, with a projected 40 million by the year 2000. 90% of infections exist in the developing world. The epidemic is currently exploding in South and Southeast Asia with about 6 million infections at the end of 1997. most of which have occurred in the past 7 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 (AFRI MS) 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 critical data to the high level characterization of the HIV-1 epidemic in Thailand. In January 1993, AFRIMS opened a Joint Clinical Research Center (JCRC) for the conduct of Phase I/II (safety & immunogenicity) 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 identify and 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 HIV pandemic is worst coincide with areas of current or potential deployment of American forces. HIV-1 is a sexually transmitted disease (STD) and hence poses a threat to forces deployed to areas where HIV-1 is epidemic. This lethal threat has been realized among United Nations (UN) forces deployed on "blue helmet" (peacekeeping) 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 clear military relevance to the development of preventive measures for the prevention of HIV-1 disease and transmission, including, especially, an effective preventative vaccine.

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. Data from animal models can be used to predict the outcome of similar events in humans. These data are reliable and can be applied to various types of research including vaccination, pathogenesis, toxicology and therapeutic agent studies. For example, one study is used to screen potential therapeutic agents for their activity against malaria. The animal model chosen for this is the mouse, one of the lowest animals on the phylogenetic scale that can be infected with malaria and then used to determine the effectiveness of new treatments. This is important not only to the military. but also to the more than 300 million people worldwide who become infected with malaria each year. In some areas, the malaria parasite is resistant to all known treatments.

However, many studies must be performed in a higher animal species. Before vaccines or drugs can be used in humans, the FDA requires that they be tested in a non-human primate model. AFRIMS is the best resource in the Department of Defense to perform this type of testing We currently have protocols approved to test two new malaria vaccines, a new Hepatitis E vaccine and a new dengue vaccine. These vaccines involve cutting-edge techniques in molecular biology for both vaccine development and vaccine delivery. The availability of modern investigative techniques coupled with the extensive animal model availability makes AFRIMS a unique facility to develop and explore the effectiveness of these new therapeutics.

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 deionized 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 FY98 are focused in three general areas: 1) preparations for HIV vaccine efficacy testing; 2) animal care and handling in support of ongoing research at AFRIMS; and 3) site maintenance and laboratory support activities.

B. Preparations for HIV Vaccine Efficacy 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.

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-l 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 Thai's. 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 Thai's

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

Thai's without HIV-1 infection

Uninfected Thai's 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 fluorescent staining and analyzed on the Facscan using Simulset software at AFRIMS.

PCR subtying

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 amplification of a 287 BP product.

d. Results

Study enrollment

Incident cases	109
Prevalent case	578
Seronegative case	108

The results presented (Tables 1-2) 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 = 109		Uninfected n = 108		Prevalent + n = 368	
	n	(%)	n	(%)	n	(%)
Age						
<20	3	(3)	0	(6)	4	(0)
20-29	98	(90)	78	(72)	325	(55)
30-39	6	(6)	22	(20)	192	(35)
40-49	1	(1)	7	(6)	41	(9)
>=50	-	-	1	(1)	15	(3)
Unk	1	(1)	1	(1)	1	(0)
Sex						
Male	95	(87)	81	(75)	381	(66)
Female	14	(13)	27	(25)	197	(34)
Home Region						
Central	5	(5)	11	(10)	151	(26)
Northeast	13	(12)	44	(41)	64	(11)
North	72	(66)	14	(13)	74	(13)
Bangkok	13	(12)	37	(34)	272	(47)
South	3	(3)	0	(0)	9	(2)
Unk	3	(3)	2	(2)	8	(1)

* 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=109)

	n	(%)
Subtype (n=73)		
E	70	(64)
B	3	(3)
EB	1	(1)
Unclassified	4	(4)
Negative	11	(10)
Indeterminant	1	(1)
Question	5	(5)
Not done	14	(13)
Estimated year of seroconversion		
1991	0	(0)
1992	29	(28)
1993	50	(48)
1994	9	(9)
1995	11	(10)
1996	5	(5)
1997	1	(1)
1998	0	(0)
Estimated time since seroconversion *		
<12 months	70	(67)
12 - 23 months	35	(33)
>24 months	0	(0)
Number of follow-up visits		
1	64	(59)
2	25	(23)
>3	20	(18)

* time from seroconversion to first enrollment

CD4 counts and percents in incident HIV-1 infections and in uninfected Thai's

A small percentage of incident cases have returned for 2 visits, therefore, a cross-sectional determination of CD4 counts by time since estimated seroconversion has been done. This indicates that CD4 counts and percents are lower in both infected and uninfected persons compared to persons in the US, but that the percent decline during the first year appears to be similar. Differences in CD4 counts and percents were also found between men and women in both HIV-infected and uninfected Thai's. Preliminary RNA viral load testing has also been performed, which demonstrates similar levels to those reported from HIV-infected persons in the US.

Assay Development and evaluation

Now the Departmental laboratories do not only continue to provide clinical laboratory assays for the natural history study but also other clinical research protocols. Those are screening of volunteers for HIV-1 vaccine trial protocol (3 sites in Bangkok [JCRC, Vaccine trial Center and Siriraj Hospital of Mahidol University]), Thai E HIV-1 vaccine protocol (3 sites in Bangkok [JCRC, Vaccine trial Center and Siriraj Hospital of Mahidol University]), QA/QC, mucosal protocol and service. From October 1997 through October 1998, clinical labs have provided the following support for the these protocols:

Laboratory	***No. samples***	***No. assays***
CBC	3,916	7,832
Immunology (flow cytometry)	1,287	1,292

The development of assays for 1998 includes growth and titration of vaccinia constructs encoding HIV genes, EBV transformation of B lymphocytes, HIV-specific cytotoxic T lymphocyte (CTL) assays, natural killer cell assays and the Chiron branched DNA assay. EBV transformation has been successful in over 85% of samples attempted. HIV-specific CTLs were demonstrated in the natural history subjects tested at AFRIMS. Branched DNA assays have been satisfactorily conducted on the natural history study subjects (range <500 copies/ml-150,000 copies/ml).

The isolation of HIV from patient monocytes was undertaken as a research project with the aim of developing a potential vaccine candidate and/or reagent.

Viral Diversity

Specimens from 12 seroconvertors from 1995-96 enrolled in the natural history were sequenced at AFRIMS. The result demonstrate relatively limited genetic variability in the V3 region compared to other HIV subtypes.

Comparison of subtype determinations

Specimens is being used in a three-way validation project incorporating an HIV serotyping assay, a nested PCR assay which differentiates HIV subtype B from subtype E (FM primers) and heteroduplex mobility assay (HMA) for genotyping.

Health Evaluation of HIV-infected and Uninfected Thai Men after Discharge from the Royal Thai Army.

a. Introduction

Understanding the natural history of HIV-1 infection in Thailand is essential in planning for phase III HIV-1 vaccine trials in Thailand where the subtype E is prevalent. The study proposed here will be a study of subjects with known dates of seroconversion and thus will generate important descriptive information about the nature of HIV disease in Thailand, its clinical signs and laboratory correlates.

Over 285 persons with incident HIV-1 infection were identified in studies conducted in the Royal Thai Army (RTA) from 1991-1995. Most of these persons have not been followed in natural history studies of HIV-1 infection. As part of this protocol, attempts will be made to locate and evaluate these subjects. Evaluation of these subjects will provide valuable insights about the natural history of HIV-1 subtype E infection in Thailand.

b. Objectives

To describe:- (1) the clinical status of persons after infection with HIV-1; (2) the distribution of CD4 counts and viral load by time since seroconversion and the relationship between CD4 counts, viral load and clinical status.

c. Methods

This is a cross-sectional study of men in Thailand who were infected with HIV-1 at a known time. The study population will consist of approximately 285 HIV infected men who seroconverted when they were in HIV-1 cohort studies in the RTA or RTAF from 1991 to 1995. A random sample of men who were not infected at the time of discharge from the RTA will be selected from the same provinces as the incident subjects and also recruited for the study. Men selected for participation will be eligible if they can provide proof of identity (e.g. Thai ID card), agree to participate, and sign a consent form.

The study will be conducted in multiple provinces in Thailand. Provinces in which at least five incident subjects reside will be targeted. Thirteen such provinces have been identified, nine of which are in the northern part of the country. Most of the HIV incident subjects were from the upper North. Therefore, the first attempt will be made to follow-up subjects from five provinces in that region. If this is found to be feasible, the study may be extended to other areas of the country.

Before the full study is initiated, a feasibility evaluation will be performed in Chiang Mai and Chiang Rai, the provinces with the largest number of incident cases. A contact form will be used to determine if subjects can be located, and to collect demographic information. A brief questionnaire will be administered to subjects contacted to determine if they would be interested in participating in a study. Based on the percentage of subjects identified, a determination will be made as to whether the study is feasible. If the study is determined to be feasible, subjects will be contacted, told about the study and asked to participate voluntarily. The field workers will not know the HIV status of the subject at the time of contact.

Study enrollment will take place at the RTA hospitals or other hospitals in the region. Subjects will be asked to come to the hospital clinics to be held on selected days.

For subjects who agree to participate in the study, the followings will be conducted at the time of the enrollment:

a. Informed consent process.
b. Enrollment and study number assignment.
c. Physical examination, including height, weight, and blood pressure check.
d. Questionnaire concerning medical history and risk behavior.
e. Pretest counseling.
f. Blood collection (20 cc).

All venous blood specimens will be drawn from the forearm in the morning and processed within 6 hours. Laboratory evaluation of all subjects will include HIV ELISA, CBC and RPR. Laboratory evaluation for seropositive subjects will include HIV Western Blot, Lymphocyte immunophenotyping, V3-peptide type-specific ELISA and sera and/or plasma viral burden.

Subjects will be provided the results of the medical screening within one month. Post-test counseling will be provided and the results of the testing will be disclosed, along with other laboratory results. Subjects who are HIV positive will be referred to a local hospital for follow-up and care. Referral can occur at any time during the study.

Hospital records will be reviewed for subjects reporting admissions since discharge from the RTA. Discharge diagnoses will be recorded on a study form. Study personnel will attempt to collect information concerning subjects who have died since discharge from the RTA. For these subjects, medical records and death certificates will be reviewed. An interview with a spouse, closest family member or other contact will be done to attempt to establish the cause of death if the subject died at home.

Analyses of these data will include

a. comparison of the general health of participants and nonparticipants
b. the percentage of subjects with AIDS and the percent with CD4 counts <500 and <200
c. the annual decline in CD4 counts
d. correlations among laboratory variables
e. differences in viral load in the first seroconversion specimens between the rapid and slow

progressors

f. comparison of behavior and psychological status among HIV infected and uninfected men

d. Results

Protocol to evaluate natural history of discharged RTA recruits aproved by WRAIR, RTA, RIHES and Thai Ministry of Public Health in the second quarter - FY98. Feasibility study to evaluate the status of men discharged from the RTA (Evaluation of HIV Infected and Uninfected Men After Discharge from the RTA) was completed in Chiang Mai. Most subjects were located and were willing to participate. Planning for the initiation of the full study and enrollment are being completed with the first enrollment expected to take place in the early part of the 1st quarter of 1999.

Evaluation of Mucosal Virology and Immunity of HIV-1 in Thailand

a. Introduction

Mucosal surfaces of the human body serve as primary barriers against infectious agents and thus are capable of generating humoral immune responses. Secretory IgA (S-IgA) is the predominant isotype of immunoglobulin prodced at mucosal sites and is the principal determinant of the mucosal response. Numberous studies have provided indirect evidence that links the antigen-specific humoral immune responses at various mucosal effector sites into a common mucosal immune system in humans. Experimental example of this include the induction of specific S-IgA in the nasal and duodenal secretions of infants after oral vaccination with live, attenuated poliovirus vaccine. Mucosal-derived antibodies against HIV-1 have been measured from a variety of sources. Archibald et al. Detected S-IgA in about 90% of parotid saliva specimens from HIV-1 infected subjects. Belec et al. Also detected IgA directed against all of major protein of HIV-1 in the vaginal washings of about 65% of infected subjects. The potential influence of mucosal response on the transmission, pathogenesis and immunity to HIV-1 have not been completely delineated. Hoever, mucosal immune responses may be protective against other infections acquired through mucosal routes. Hence. These are important parameters to monitor in both naturally infected and vaccinated individuals. To this end, ongoing and future clinicl trials of HIV-1 candidate vaccines will usually include complete assessments of mucosal immune responses from multiple sites in each individual in order to evaluate the relative ability of different products and regimens to elicit local antibody.

b. Objectives

The purpose of this study is to evaluate the virologic and immunologic parameters of HIV-1 in mucosal and systemic components of Thai subjects.

c. Methods

Specimens including blood, nasopharyngeal washings, endocervical secretions, vaginal washings, semen and urine were collected from HIV-1 infected and non-infected Thai males and females population. These samples were evaluated in the laboratory for HIV-1 serologic testing, complete blood count, immunophenotyping, HIV subtyping , the total and HIV-1 specific IgG, IgA and S-IgA, The measurement of HIV-1 viral burden in blood and HIV-1 peripheral blood monnuclear cell co-culture in semen and vaginal wash were also performed. The results of these studies would be analyzed to compare the intra- and inter- subject antibody responses between and within compartments, to find out the influence of epidemiologic and clinical factors on humoral resposes in mucosal compartments, to compare the seminal or vaginal cell culture results with HIV-1 RNA levels and antibody measurements and to evaluate the effects of HIV-1 subtype on cell culture results, HIV-1 RNA levels, and antibody measurements.

d. Results

The MOPH ethical review committee approved the mucosal protocol on April 28 1998. The protocol was implemented in June 1998. To date, 56 subjects have been enrolled. The ration of uninfected to infected is 2/1.

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. One of these is completed and the other continued through FY98.

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 Gahn) . 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 Khirl Khan is the southernmost province of the Central region. Fort Thanarat, the major RTA installation in the province has conscripts from geographically diverse backgrounds. Conscripts who arrive for service in May generally come from the Central or Southern provinces, while those who arrive in November are drawn from the Northeast. Fort Thanarat was chosen because it had a large recruit population, increasing prevalence, predominantly non deploying units (to simplify follow-up), and a single large hospital responsible for care. It's geographically diverse population also permitted exploration of regional differences in epidemiology and behavioral norms. 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 Fort Thanarat, Prachuap Khiri Khan province, 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. using a non-randomized, quasi-experimental design. The incidence of HIV-1 in the recruits, over all and in the two intervention groups, will be determined, along with changes in knowledge and behavior over time. At the end of the follow-up period, subjects will complete a questionnaire to assess attitudes towards participation in vaccine trials. As a service and incentive to the conscripts, Hepatitis B immunization is being offered, along with treatment of prevalent cases of syphilis. Routine follow-up and care is provided for HIV seropositive participants in this study. The HIV care and behavioral interventions will be adapted by the fort hospital and continued after the study is completed.

d. Results

3011 seronegative recruits were enrolled for incidence follow-up, 88.7 % were available for follow-up at 24 months. The overall incidence was 0.43%/100py. During the study period, CSW patronage decreased from 24.8% to 15.5% ($p<0.05$) and consistent condom use with CSWs increased from 59.7% to 76.4% ($p<0.05$). Of the seroconverters, 89.1% participated in a program for prophylaxis and treatment for HIV-related infection. Of the HBV-naïve, 59.3% presented for first Hepatitis B vaccination and 90.2% of these vaccinees received a full 3 doses course.

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. The study is scheduled to be completed in April 1997.

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

Between September 1995 and February 1996, 1901 eligible persons were asked to participate in the study. Thirty percent of eligible men (371/1238) and 24% of women (161/663) agreed and were enrolled into the study. Among the 532 person who enrolled in the study, the HIV-1 seroprevalence was 3.4%. History of an ulcerative SI'D and lifetime CSW partners were associated with HIV-1 infection among men. There were no statistically significant risk factors identified for women. Follow-up at the second and third study visits has been 70-80%. To date,

4 incident HIV infections have occurred in study population; all among men. The over all incidence was 1.4%/100py. Data analysis and preparation of manuscript is ongoing.

Incidence of HIV-1 Infection Among Women Attending Family Planning Clinics in Rayong Province, Thailand

a. Introduction

HIV poses a significant threat to Thai and U.S. military personnel. Therefore, the Royal Thai Army and the U.S. Department of Defense are supporting a research and development to minimize the impact of HIV on military readiness by monitoring the spread of HIV infection in both military and civilian components around the world and, through the development of vaccines and other countermeasures, to prevent infection and infection sequelae.

Plans for AIDS vaccine research in Thailand have included preparations for phase I/II and for large phase III efficacy trials. Cohorts in Thailand either have been investigated for their potential for phase III efficacy studies, or have been developed for other purposes by several groups. In this feasibility study, we propose exploring
cohort development within family planning clinic attendees in which women return every three months for a refill of oral contraceptives or an injection of Depo-Provera. This population has several advantages that might prove it a successful cohort in terms of follow-up.

In addition to evaluating HIV-1 incidence in this cohort, changes in risk behavior and incidence of other STD's will be evaluated as well as follow-up rates. Attitudes and motivation for participation in HIV vaccine trials will be investigated by a questionnaire administered at the end of the study.

b. Objective

To determine (1) the incidence of HIV-1 infection in a cohort of women who attend family planning clinics; (2) the rate of follow-up during the one year study period in this cohort; (3) the subtype and further characterize HIV isolates from seroconvertors in the cohort; and (4) assess willingness to participate in HIV vaccine trials.

c. Methods

Three health centers in Rayong Province will be used for recruiting volunteers in this study including: Nernprah Health Center, Pae Health Center, and Tapong Health Center. Women who attend the family planning clinics will be asked to participate in the study when they come in for their quarterly follow-up or as a new patient to the clinic desiring birth control .

A person will be eligible for participation in the study if she:

-Is participating in a family planning program at one of the designated health centers.

-Is at least 20 years of age.

-Can read or understand Thai.
-Voluntarily agrees to participate and signs a consent form.
-Health status such that volunteer is able to be prescribed oral contraceptives or an injection of Depo-Provera.
-Plans to remain in Rayong for at least 1 year on enrollment.

Recruitment will take place over a 4 month period or until at least 500 subjects are enrolled and will be terminated earlier if 1000 subjects are enrolled before that time. At the initial visit, 10 ml. of blood will be drawn for HIV-1, syphilis and hepatitis B testing and for storage. Data collected at the time of enrollment will include demographic data, general medical data, and perceived behavioral risk factor data.

Optional return visits will be scheduled two weeks after the initial visit and after each subsequent visit for notification of test results. Participants who are found to be eligible for hepatitis vaccine will be offered vaccination. Those who have positive syphilis serology and have no history of recent treatment will be referred for treatment to the Rayong Hospital. HIV counseling will be provided before every blood draws and test results. HIV seropositive subjects will be referred to Rayong Hospital for initial work-up of their infection. No further follow-up will be provided as part of the study.

All participants will be asked to return at 6 month intervals for one year. Participants who are found to be HIV positive will receive further follow-up as part of this study. Venous blood will be drawn for HIV-1 by the study/clinic staff. Interviewed questionnaire will be administered to collect data on perceived risk to HIV-1 and any diagnosis of an STD in the subject or their sexual partner/partners since the last visit. At the last visit, a standardized questionnaire about knowledge of and interest in vaccine trials will be administered.

For seropositive subjects, additional blood (approximately 15 ml total) will be obtained for lymphocyte immunophenotyping and DNA PCR (seroconvertors only). CBC and immunophenotyping will be performed at Rayong Hospital. PCR on seroconvertor subjects will be done at AFRIMS.

Blood for PCR will be collected in a citrate leukoprep tube. The tube will be centrifuged and transported to AFRIMS on insulated ice packs and processed within 24 hours of receipt. The resulting plasma aliquoted in 1ml aliquots and stored at -80°C. The PBMC pellet will be aliquoted as 2.5 X 10^6 cells/vial in 1 ml of freeze media and cryopreserved at -140°C.

Residual serum, not required for immediate screening, will be stored in 0.5 ml aliquots at -20° C and shipped to AFRIMS for long term storage at -80° C.

Analyses of these data will include calculation of annualized incidence overall. Analysis of variance (ANOVA or appropriate nonparametric tests) will be used to examine how different levels of willingness to participate in vaccine trials are related to social discrimination, benefits to self and demographic variables.

d. Results

Protocol has been approved by U.S., Thai RTA and MOPH Human Use committees in November 1997. Enrollment in the protocol was begun on 26 February 1998 and was complete in 11 june !998. We were able to enroll the maximum number of volunteers sought (1002). Data entry is on-going. The HIV-1 prevalence late is 3.9% (39 seropositive/1002 enrolled)(6.9% in the 20-29y/o group). Approximately 400 volunteers have completed the first 6 month follow-up visit, incidence data is currently pending.

Community-Based Cohort Study of HIV-1 Incidence in Sattahip, Chonburi, Thailand

a. Introduction

Plans for AIDS vaccine research in Thailand have included preparations for phase I/II and for large phase III efficacy trials. In this feasibility study, we propose community-based cohort development in two communities in the subdistrict of Sattahip in Chonburi, Thailand: Phlutaluang and Chong Samaesan. Chonburi is a province located southeast of Bangkok on the eastern seaboard.

While the size of the communities evaluated in this protocol may not be adequate for phase III trials, this concept could be enlarged and adequate numbers enrolled from a community-based cohort. Calculations have been made to determine the cohort size needed for phase III efficacy studies. AFRIMS has and is exploring a variety of cohorts. Because vaccine efficacy trials should be able to start in the year 2000, several cohorts need to be explored simultaneously in order to ensure that a cohort is available when vaccines are ready for phase III trials.

b. Objective

To determine:- (1) the baseline Human Immunodeficiency Virus, Type I (HIV-1) prevalence and the HIV-1 incidence in persons 20-49 years of age in a community-based study in Sattahip, Chonburi, Thailand; (2) participation rates and differences between participants and non-participants; (3) the follow-up rates during the study period; (4) attitudes toward participation in phase III HIV vaccine trials; (5) behavioral changes in the participants during the study period; and (6) HIV subtypes among HIV-1 infected persons in the cohort.

c. Methods

This study will be a prospective descriptive cohort study and will be conducted in two communities in the province of Chonburi: Phlutaluang and Chong Samaesan. Prior to recruitment, information will be provided to community leaders. Following this, a local health staff team will perform a house to house survey to identify eligible volunteers by using a screening form. If an eligible community member is interested in the study, he/she will be referred to the study site for formal enrollment.

A person will be eligible for participation in the study if he/she:

- Is 20-49 years of age.
- Is a Thai citizen.
- Voluntarily agrees to participate and signs a consent form.
- Plans to remain in the community for at least 2 years.

Recruitment will take place over a 4-5 month period.

d. Results

The protocol for the community based cohort in Sattahip has been approved by both the RTA and MOPH. Preliminary meetings with local MOPH officials are on-going and implementation of the protocol is expected to begin during the next quarter.

3. HIV-1 Vaccine Testing

Screening and evaluation of potential volunteers

a. Introduction

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 were 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 50 days in advance of actual trial approval allows for a more rapid implementation and enrollment phase for each vaccine trial.

Information from this protocol is being used to guide future recruitment strategies. Additionally information on normal lab values obtained in screening for the RV99 protocol has been useful in the design inclusion and exclusion criteria for future HIV research protocols in Thailand.

The protocol was amended to include the two new TAVEG sites (Vaccine Trial Centre, Faculty of Tropical Medicine and Siriraj Hospital, both of Mahidol University) and to it more flexible as a screening tool for various vaccine protocols. Some examples: the age range was changed from 20 - 50 to be age 18 or older. The requirement for Thai nationality was removed, some of the specifics about lab assays and were made more general to allow flexibility in future studies, as were the descriptions of the sequence of procedures at each visit. A section was added to allow compensation to be paid for the last screening visit for those volunteers who are found to be eligible for the upcoming vaccine protocol.

b. Methods

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

c. Results

This protocol, approved in January 1995, enrolled 107 volunteers between 1 August 1995 and 14 December, 1995 to screen potential volunteers for RV99 protocol ("A phase I Trial of Biocine HIV SF2 gp120/MF59 Vaccine in Seronegative Thai Volunteers"). Enrollment recommenced on 29 September, 1997 to screen for RV114 vaccine protocol (A phase I/II, Double-blind, placebo-controlled Study of Chiron HIV Thai E gp120/MF59 Vaccine Admisnistered Alone or Combined with the Chiron HIV SF2 gp120 Antigen in Healthy HIV-Seronegative Thai Adults). 611 volunteers (VTC 151, Siriraj 123, AFRIMS 132, RIHES 203) have been enrolled at the four TAVEG study sites.

The preliminary finding of these volunteers are the following:

- Continue high altruistic motives for participation.
- Negligible results from mass media. Majority of volunteers were either recruited from through community outreach or referral by study staff or study volunteers.
- Demographic differences between volunteers enrolled in Bangkok and Chiang Mai in that Chiang Mai volunteers were more likely to be married, have lower education and personally know someone with HIV.
- Approximately 65% of volunteers completed screening successfully and were enrolled in RV114 vaccine protocol.
- Compared to Bangkok sites, Chaing Mai site had more screening failures as a result of more withdrawal of consent, failing test of understanding and medical / laboratory abnormality.
- Only 3 screening volunteers were found to be HIV infected. Majority of medical / laboratory exclusions were due to hypertention, elevated liver function and microscopic hematuria.

Phase I trial of Biocine HIV SF2 gp12O/MF59 vaccine

This double-blind, randomized, Phase I study evaluates the safety/tolerability and immunogenicity of the BIOCINE Human Immunodeficiency Virus (HIV) SF2 gpl2O/MF59 Vaccine at the dose of 50ug in two immunization schedules. The study population consisted of fifty-two HIV-1 seronegative, healthy Thai adults enrolled from the community, twenty-six at AFRIMS in Bangkok and twenty-six in Chiang Mai. Each site had one drop out who was replaced, so a total of 54 volunteers were enrolled.

The final regular visit occurred 18 September 1996. Compliance was 100% for each visit at each site. Each subject was asked to return for three follow-up visits at 6-month intervals. The last one will occur on 29 November 97. The compliance rate (overall for 2 sites) was 96% of volunteers at first follow-up visit, 84% at the second, and 74% at the final (18-month) follow up visit.

Results to date indicate that the vaccine is safe. It induces no significant systemic toxicity or local reactogenicity; the safety profile of the vaccine in vaccinated Thais similar to that seen in volunteers who received this product in clinical trials in the United States. Binding and neutralizing antibodies were elicited, as were lymphoproliferative responses. These immune responses appeared greater in magnitude with the third dose at 6 rather than 4 months.

Manuscript regarding recruitment and enrollment has been accepted for publication and represent the first published evaluation of HIV vaccine recruitment in the world. Manuscript regarding reactogenicity and immunogenicity is in preparation. Attempts were made to contact all 52 volunteers; 39 were contacted by phone or in person and 10 were contacted by letter. (Three had moved, with one now living in Australia.) Thirteen had had an HIV blood test in the preceding year (10 of these were for blood donation), all of which were negative. Three subjects had been hospitalized during the prior year, two for motor vehicle accidents and one for influenza. One subject is receiving treatment of hyperthyroidism. All other volunteers describe their health as the same as or better than that existing prior to their participating in the trial. There have been no volunteers developing HIV infection during the course of an AFRIMS HIV vaccine trial, neither in this completed trial nor in a subsequent ongoing trial.

A Phase I/II Double -blind, Placebo Controlled of Chiron HIV Thai E gp120/MF59 Vaccine Administers Alone or Combined with the Chiron HIV Sf2 gp120/MF59 Vaccine in Healthy HIV-seronegative Thai Adults

a. Introduction

There is considerable known genetic variation among HIV-1 strains isolated from differing geographical locations worldwide. This variation is especially apparent in immunologically active envelope epitopes that are associated with *in vitro* antibody-induced neutralization, ie, specific neutralizing responses induced by North American HIV-1 strains are not capable of cross-neutralizing all divergent HIV-1 strains.[2] However, the importance of vaccine-induced neutralizing antibodies, or any other specific immune effector, for *in vivo* regulation and

prevention of HIV-1 in humans is uncertain. Therefore, evaluation of candidate vaccines that are composed of antigens commonly circulating in a given location and population and that have been demonstrated safe and "immunologically active" in preclinical studies is a reasonable strategy. HIV-1 isolates routinely recovered from newly infected individuals in Thailand are of envelope genotypes B and E, with E viruses predominating.

The Chiron recombinant subunit HIV-1 SF2 gp120 antigen is derived from the SF2 gpl20 antigen isolate of HIV-1, which is a member of the B genotype. This genotype is prevalent in Northern America, Europe, Haiti, and is present in other countries such as Thailand. This gpl20 antigen is expressed in genetically engineered mammalian Chinese hamster ovary (CHO) cells. Since 1988, over 800 subjects have participated in the evaluation of the Chiron HIV-1 SF2 gpl20 Antigen/MF59 Vaccine program, including 70 newborns of HIV-infected mothers. A total of 13 phase I trials and one phase II trial were conducted in which the Chiron vaccine was tested alone or in combination with other HIV vaccines in the US and in Thailand. Results from these studies showed that the Chiron SF2 gp120/MF59 Vaccine is very safe and well tolerated. It induces neutralizing antibodies against several genotype B viruses most notably the homologous SF2 gp120 antigen strain. These antibodies persist for a 6-month period at minimum, and can be boosted. In addition, this vaccine induces an HIV envelope specific cellular immune recognition and proliferation. The vaccine is being tested in a phase I trial involving 52 healthy HIV seronegative adult Thai subjects at Armed Force Research Institute of Medical Sciences (AFRIMS) in Bangkok and at Research Institute for Health Sciences (RIHES) in Chiang Mai (Protocol V6P17). Preliminary data indicate that the vaccine injections were also well tolerated and that the safety profile is not significantly different from the one observed in US subjects. These characteristics, and the observation that this vaccine induces protection in the chimpanzee model, represent a minimal set of criteria that justifies further evaluation of the vaccine in Thailand.

However, the most prevalent HIV envelope genotype in Thailand is group E. Therefore, Chiron has recently focused its effort on cloning and manufacturing a Thai E gp120 antigen, which is derived from the modified Chiang Mai CM 235 HIV strain. This antigen is also produced in a genetically engineered CHO cell system. Animal experiments in guinea pigs and baboons show that this Thai E gp120 antigen induces gp120 antibodies against the homologous strain and the heterologous SF2 strain. They also show that addition of the Thai E gp120 antigen to the SF2 gp120 antigen did not adversely effect the immunogenicity of the SF2 gp120 antigen.

The MF59 adjuvant is an oil-in-water emulsion that has already been administered to approximately 6,000 individuals in various phase I, II, or III Chiron vaccine trials. These trials have included studies of the MF59 adjuvant emulsion administered in the absence of antigen or administered with cytomegalovirus , influenza, herpes, or HIV antigens. The results indicate that MF59 formulation is generally well tolerated, with transient pain and tenderness at the injection site reported by most subjects.

The present study (V26/6P1) will evaluate the safety and immunogenicity of the Thai E gp120/MF59 vaccine administered alone or in combination with the HIV-1 SF2 gp120 antigen in

healthy Thai adult subjects. The goal of this study is to provide the necessary information to choose the best antigen combination and dose for a potential candidate HIV vaccine that might be evaluated in an efficacy trial in the Thai population. This will be the first time that the Thai E antigen is administered to humans. For additional information, please refer to the SF2 and Thai E gp120 Investigator Brochures.

b. Objective

- **Safety Objective :**
  To evaluate and compare the safety of three doses of Chiron HIV Thai E gp 120/MF 59 Vaccine (25, 50, 100 μg) alone or combined with one of two doses of the Chiron HIV SF2 gp120 antigen (25 and 50 μg) in healthy, HIV-1 seronegative Thai adults.

- **Immunogenicity Objective :**
  To evaluate and compare the immunogenicity of the three above-mentioned doses of the Thai E gp120/MF 59 Vaccine when given alone or combined with one of two doses of SF2 gp120 antigen and to evaluate potential interactions between antigens. If there is no meaningful interaction between antigens, comparisons will be made among the Thai E gp120 antigen dose groups and the SF2 gp120 antigen dose group.
  These comparisons will allow for the selection of a vaccine candidate anticipation for a future efficacy study.

c. Methods

After a 12 subject open-label trial (Part A) has proceeded through two injections at two sites, the randomized, double-blind, placebo-controlled, phase I/II trial (Part B) will be initiated. Four collaborating centers in Thailand (AFRIMS, RIHES, Mahidol University Vaccine Trial Center and Siriraj Hospital) will evaluate the safety and immunogenicity of the Chiron HIV Thai E gpl2O/MF59 Vaccine at doses of 25, 50 and 100 μg, alone or combined with the Chiron HIV SF2 gp120 Antigen at doses of 25 or 50 μg, administered at 0, 1, and 6 months in healthy Thai volunteers. Ninety-two volunteers at each center will be randomly assigned to 1 of 9 antigen groups (n=8) or to placebo (n= 20). The placebo is a vehicle material containing MF59 alone. Part B is double-blinded, and a randomization code will be generated for each subject assignments.

Part A

Open-label Phase*

Antigen/Dose	Site	N
Thai E gp120 (25 μg)/MF59	RIHES	3
Thai E gp120 (25 μg)+SF2-gp120 (25 μg)/MF59	VTC	3
Thai E gp120(100 μg)/MF59	VTC	3
Thai E gp120(100 μg)+SF2-gp120 (50 μg)/MF59	RIHES	3
		12

* Dose combination will be given sequentially, separated by at least 48 hours

Part B

Double-blinded Phase

Thai E gp120 antigen (μg)	SF2 gp120 antigen (μg)	N (per site)	N (per site)
25	0	8	32
50	0	8	32
100	0	8	32
25	25	8	32
50	25	8	32
100	25	8	32
25	50	8	32
50	50	8	32
100	50	8	32
0	0	20	80
	TOTAL	92	368

Prior to enrollment and at each study visit after volunteers will be counseled regarding avoidance of HIV exposure. A registration sheet will be used to obtain identifying and demographic information about each volunteer. After completion, this form will be filed separately from case report forms (CRFs), which will be identified by subject number only. An assessment of absolute exclusion criteria using a questionnaire and interview questions, a medical history, physical examination, and blood sampling will be done. Ultimate eligibility for the trial will depend on results of laboratory tests, clinical evaluation, and acknowledged high-risk behavior.

Subjects will be observed in the clinic for at least 30 minutes postimmunization. At 30 minutes, the subject will be evaluated for any signs of symptoms of local or systemic reactions. The subject will be instructed on measuring his/her temperature and noting any symptoms at 6 hours postimmunization. A subject diary card will be supplied for recording temperature measurements and any symptoms of local or systemic reactions for 7 days following immunization. The diary card will be collected at the next scheduled visit following each immunization, and kept in the subject's file. All subjects will be contacted by the study staff within 24 to 72 hours postimmunization by telephone or by home visit to assess any symptoms reported. Evaluation by an investigator will be scheduled if significant symptoms are reported. All adverse events occurring up to 30 days after the third injection will be collected. After that, ie, after visit 6 at week 28, only adverse events that are serious and/or necessitate a physician's visit or a prescribed medication will be collected. All collected adverse events will be monitored until resolution. In addition, clinical laboratory parameters will be evaluated.

d. Results:

Open label trial was started on November 10, 1997. Extensive monitoring of the first 12 volunteers for the open label is conducted. The second immunization of the twelfth volunteer was on December 18, 1997. Analysis of reactogenicity data and clinical laboratory data was on December 24, 1997. The investigators concluded that this vaacines were safe. The data was send to DSMB for review and approval for proceeding to part B.

Approval for the initiation of the Double-blind phase of the trial was given by the DSMB after reviewing the safety data from the open lable phase completed last quarter. The opening Ceremony attended by RTA, MOPH, AFRIMS, US Embrassy, WRAIR dignitaries was held in January.

Enrollment into the trial was completed and closed in early July At the end of September 1998 approximately 70% of the volunteers had completed their 3rd and final vaccination at all 4 sites. There have been three drop-outs from the trial and six serious adverse events (SAE). None of the SAEs were related to the vaccine. Reactogenicity to vaccine/placebo appeared mild to moderate. The most common complaints were headache for 1-2 days, mild malaise, myalgia and arthralgia, pain at injection site varies from mild (most cases) to severe (rarely). Rashes occurred a few times, all resolved after a few days.

The last volunteer enrolled received the first injection of vaccine on June 23, 1998. The last injection will be given on December 8, 1998. Two blood draws, for follow up of HIV status only, will occur at 6 & 12 months following the study termination.

A monitoring visit was conducted by the sponsors (Chiron and WRAIR/HJMF) in September 1998. All sites were monitored with 100% of the records monitored. The visit when exceptionally well with only minor errors found.

A Refresher Seminar for site nurses was held in September in Bangkok. Attendance was nearly 100% including nurses from RIHES in Chaing Mai. Sessions included an overview of the Prime Boost Vaccine, HIV counseling, CRF completion, BCLS, etc. All the nurses enjoyed the presentations and reported that information presented was extremely useful and informative.

QA/QC programs

Development of Quality Assurance program in all laboratories. Training sessions on the SOPs and QA/QC issues have been held for lab personnel at all sites. AFRIMS was registered with the College of American Pathologists in April 1998 to receive proficiency panels pertinent to our clinical assays. To date, panels have been received for CBC, viral load and serum chemistry. Monthly Streck Statistical Analysis reports from CD Chex data submitted by the AFRIMS and RIHES sites is ongoing with favorable results reports being received. Data from the first UK/NEQAS panel were submitted and the initial result report is due in July 1998. UK/NEQAS

panels are provided six times per annum. Five quarterly AFRIMS proficiency testing (PT) panels have been distributed since May 1997. Results reports for this period were consistent for all sites involved; as described in the PT Panel Distribution Annual Report. Two WRAIR HIV serological proficiency testing panels have been received in Thailand and distributed to appropriate sites within the country. Result reports are consistent for all sites involved

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.

In 1996, serotyping of all HIV positive sera was initiated using a V3 peptide ELISA. In addition, a comparison of serotypes in a random sample of recruits from each regions in 1992 and 1995 was performed.

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

c. Results

Trends in seroprevalence in the RTA

Demographic information was collected on young men entering service with the Royal Thai Army nationwide and is merged with routine serologic HIV data collected by the RTA. The recruits were bled at entry into the RTA (every November and May) and sera were tested for

HIV by ELISA (confirmed by Western blot). In 1997, serotyping of all HIV positive sera was initiated using a V3 peptide ELISA.
Data from this study is analyzed to evaluate trends in nationwide seroprevalence.

Trends in seroprevalence in the RTA

HIV-1 Seroprevalence (%) by Region of Service in the RTA and Year

Region	**1990**	**1991**	**1992**	**1993**	**1994**	**1995**	**1996**	**1997**
Central*	1.3	2.2	2.9	3.0	2.6	2.5	2.0	1.8
Bangkok	1.2	2.8	3.3	3.2	2.9	2.6	1.7	2.2
Northeast	0.9	1.8	2.4	2.6	2.5	1.6	1.6	1.1
North	6.1	6.5	7.5	7.3	5.0	3.4	3.3	2.5
South	1.6	2.2	2.7	2.8	2.2	2.1	2.1	2.6
Total	**1.9**	**2.9**	**3.5**	**3.6**	**3.0**	**2.4**	**2.1**	**1.9**

* Bangkok not included

Serotyping

Over 95% of prevalent infections in 1997 were subtype E.

C. 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 seventeen active animal-based protocols. At any given time, we house about 5,000 animals of 10 different species, including three non-human primate species and four rodent species.

b. Results

During FY98 care and handling were provided for 2,950 animals in support of 12 research studies (Department of Entomology = 3, Immunology = 2, Virology = 2, Vet med = 3, Retrovilogy = 1, Enteric Diseases = 1).

Animal utilization, AFRIMS, FY98

Animal Species	Number of Animals
Rhesus monkey	412
Cynomogous monkey	116
Mice	2,222
Hamster	158
R. rattus rat	34
Sheep	4
Geese	4

D. 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. The glassware section is also responsible for the daily production of sufficient quantities of pure deionized water for use in laboratory assays. The glassware section also invariably complies with AFRIMS safety regulations to make sure that all hazardous waste materials be discarded in a proper place for environmental protection.

b. Results

During FY98, the glassware section received 53,700 items of glassware for cleaning and decontamination. They distributed 36,330 items for use by various departments at AFRIMS. All hazardous waste materials after decontamination either by chemical treatment or under sterilization process will be dumped in the proper place.

Average daily glassware use, AFRIMS, FY96

Department	Flasks	Bottles	Beakers	Cylinders	Tubes	Pipettes
Virology	60	120	60	30	1,400	300
Immunology	10	30	20	10	300	
Entomology	10	10	20	10		
Retrovirology	5	15	15	10		
Bacteriology	10	10	10	10	800	100
Retrovirology	10	10	20	5		

III. CONCLUSIONS

A. Preparations for HIV Vaccine Efficacy Testing

1. Natural History Study

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 follow-up 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. Two new protocols regarding the natural history of HIV infection were implemented during this fiscal year. Information obtained from these protocols will help to better understand the pathogenicity of HIV infection inThailand.

2. Cohort Studies

The intensity of effort and resources which such undertakings demand has only become apparent with experience. Three cohort feasibility projects have been implemented. Study of a civilian cohort (STD clinic attendees) and a military cohort were completed. While these two study yields valuable data, they also demonstrated that neither a STD clinic attendees cohort nor a military cohort was optimal as the potential study population for a phase III vaccine efficacy trial. The study of women in MOPH family planning clinics is on-going in Rayong province. Enrollment was excellent. Cohort development of community based cohort in Sattahip will begin in FY99.

The single-most important ingredient in successful cohort projects is a solid base of support and trust within the collaborating institutions. The Royal Thai Ministry of Public Health (MOPH) and the network of ministry sponsored hospitals and clinics have been most cooperative at all levels of cohort development. Cohort development within MOPH facilities and with civilian subjects, have required considerable efforts to establish working relationships with key individuals, including the Director, Department of Communicable Disease Control, the Director of the Division of AIDS and with numerous ministry officials at province, district and community levels. In the case of the RTA, success was based upon relationships built with hospital and based commanders, and support from the central command.

3. Phase I/II Vaccine Trial

The first phase I/II trial of an HIV vaccine (rgp120) was completed with vaccine found safe and immunogenic in Thais. Manuscript is almost ready for submission for publication. A large phase I/II trial of a subtype E HIV subunit vaccine (rgp120) is on-going. Enrollment to the trial was completed. There was no serious adverse events related to the vaccine. A monitoring visit

was conducted by the sponsors (Chiron and WRAIR/HMJF) in September 1998. All sites were monitored with 100% of records monitored. The visit went well with only minor errors found.

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. Serotyping defines 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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PERSONNEL ASSIGNED UNDER CURRENT AGREEMENT

Vet. Med.

No.	*Name*	*Surname*	*Position*
1	Komdej	Kongsunarat	Lab Animal Research Supervisor
2	Niyom	Sornchan	Supervisor Monkey Section
3	Phongsak	Maneerat	Supervisor Assistant
4	Bumrung	Chaikwang	Supervisor Assistant
5	Manas	Suphasri	Supervisor Rodent Section
6	Pakdee	Chuenchom	Supervisor Assistant
7	Suchin	Poolgird	Supervisor Assistant
8	Samran	Kongsua	Animal Care Taker Technician
9	Ampai	Bhudthongchai	Animal Care Taker Technician
10	Thonglor	Detkokao	Animal Care Taker Technician
11	Sawang	Sripakdee	Support Supervisor Section
12	Phatcharaphon	Jaikla	Operator Equipment
13	Samruay	Jecksaeng	Operator Equipment
14	Dechmongkol	Onchompoo	Operator Equipment
15	Manop	Pooyindee	Operator Equipment
16	Choosri	Sangsri	Guard
17	Komgrit	Ekkachart	Admin. Assistant
18	Jarin	Keawjarat	Lab Animal Research Assistant
19	Sarawut	Komjalern	Lab Animal Research Assistant
20	Anchalee	Tungtang	Lab Animal Research Assistant

Glasswares Worker

No.	Name	Surname	Position
21	Sawadi	Boonnak	Glasswares Worker Supervisor
22	Charan	Kajeechitr	Glasswares Worker
23	Thongchai	Duangkaew	Glasswares Worker
24	Boonthum	Jamjang	Glasswares Worker
25	Komson	Boonnak	Glasswares Worker

Administration

No.	Name	Surname	Position
26	Sutthida	Srijan	Admin. Clerk
27	Weerasak	Yeephu	Computer Technician
28	Sompol	Boonnak	Computer Technician
29	Daungjai	Lumson	Data Entry
30	Barnyen	Permpanich	Nurse
31	Ratchada	Thipwong	Medical Technician
32	Nipat	Promchart	Air-Conditining Repairman
33	Russama	Jittawisuthikul	Admin. Clerk
34	Thongsuk	Munmuenpom	Driver
35	Somsak	Sangsri	Driver
36	Somchai	Putsang	Driver
37	Pattrapan	Jullasing	Information Technologist
38	Puwanai	Sangsri	Audio-Visual Assistant

HIV Personnels

NO.	*Name*	*Surname*	*Position*
1	KRITIKA	SINGHARAJ	ADMIN. ASSISTANT
2	NARONGRID	PONGPAKDEE	DATA ENTRY
3	NUCHAREE	THONGSEN	CHIEF OF DATA ROOM
4	PLOYPAILIN	KHLAIMANEE	LOGISTIC ASSISTANT
5	YAOWALUX	KITKUNGWAL	BAA SECRETARY
6	WAREEPORN	WONGBOWONNAN	PI. SECRETARY
7	WONLANA	JAIDEE	DATA ENTRY
8	VIROJ	YAMUTHAI	DATA ENTRY
9	WISUT	LOKPICHART	PROGRAMMER
10	SITHINAN	BUNYATUB	DATA ENTRY
11	SUCHAT	THEPSANAN	DATA ENTRY
12	SUPIN	PANKOTE	DATA ENTRY
13	ORANUCH	SUPAPYAN	SPECIAL PROJECT TECH
14	APORN	CHITSUNTHORNRAT	SPECIAL PROJECT TECH
15	KORNCHANOK	PANJAPORNSUK	TECHNICIAN
16	KAMPOL	PUAPUEN	RESEARCH ASSISTANT
17	VINAL	KANEECHIT	RESEARCH ASSISTANT
18	SIRIVAJRA	EKAPIRAT	MEDICAL TECHNOLOGIST
19	SUTCHANA	TABPRASIT	TECHNICIAN
20	SUCHAT	CHUANGPHO	CLEANER / MESSENGER
21	APICHAT	SUDATHID	TECHNICIAN
22	ATHAYA	RUANGPHUENG	TECHNICIAN