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PRINCIPAL INVESTIGATOR: MG Gobchoke Puavilai

CONTRACTING ORGANIZATION: Armed Forces Research Institute of Medical Sciences/
Royal Thai Army Medical Component
Bangkok 10400 Thailand

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Medical Component
Bangkok 10400 Thailand

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Gobchoke Puavilai

PI - Signature Date 17 Feb 2000

MG Gobchoke Puavilai, MD
Principal Investigator
Royal Thai Army, AFRIMS
Bangkok 10400, Thailand

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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

The World Health Organization estimates that HIV-1 currently infects 30 million people (including 1.1 million children) worldwide, and over 5 million have developed AIDS . While recent advances in antiretroviral therapy have improved outcome for HIV-1-infected patients in developed countries, new HIV-1 infections occur at a rate of approximately 16,000 per day, 90% in less developed countries where anti-retroviral therapies will have limited availability

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 FY99 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

1a. 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. Accomplishment

a. Enrollment:

FY 99 = 29 cases

Prevalence Cases = 25
Incidence Cases = 4

Total Enrollment = 866

Prevalence Cases = 704
Incidence Cases = 162

b. Assay Development: This project continues to provide an excellent source of samples for the development and optimization of assay systems in the various AFRIMS laboratories including CTL, NK, ADCC, and neutralization assays specifically for the subtype E. The development of these assays have and continue to be critical in the program's Vaccine evaluation program in the study of immunological and virological correlates of infection and progression of disease in these studies.

c. Serotyping: Incidence and prevalence cases from this project continue to show the narrow diversity of subtypes (B and E) being transmitted in Thailand. Further, this indicates that Thailand continues to be an ideal location for the evaluation of vaccine candidates. During this year a panel of 176 cases were examined specifically for dual and recombinant

B/E infections. This work continues although preliminary evidence indicates that a potential B/E recombinant virus has been isolated from at least one case where a discordant serotype vs. genotype occurred.

d. The sequence for the subtype E prot gene has been determined using a group of 20 patient viruses for this project.

e. A panel of cases (Initial visit, CD4 > 450; F/U > 3 yr) has been identified from this project that will be used to determine the immunologic and virologic differences between disease progressors and nonprogressors.

1b. 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. Accomplishment

Enrollment of this protocol was completed in August 1999. Of the 570 young Thai men sought during the course of the study, 404 (79.8%) were located throughout Thailand.

Total Men Sought	505
Total Men Enrolled/Deceased	405
Total Enrolled	317
+ HIV	123
- HIV	194
Total Deceased	81
+ HIV	74
- HIV	7

The data from a cohort of these men has been analyzed. This cohort comprises men enrolled from the upper northern provinces of Thailand. Of the 205 men contacted from this area 200 (97.6%) were identified and of these:

157 (78.5%)	Found alive and enrolled
34 (16.5%)	Found alive but not enrolled
9 (4.5%)	Found deceased

The major cause of death in this cohort was motor vehicle accidents (5) and the remaining causes were cirrhosis, electrocution, heart failure and suicide attributable to HIV status (each 1 case). Sixty-five present were married, 98% were employed as either farmers or labors and 80.9% had at least 9 years of education. Of those that were HIV – at time of discharge, 5 had seroconverted. One of the converters had committed suicide due to conversion and the 4

remaining were asymptomatic. Thus, the incidence within this group was 0.62/100 PY. This incidence is comparable to other recent studies of HIV incidence in other cohorts and confirms the low incidence of HIV in young Thai men. The probable route of infection of these incident cases was heterosexual (4) and IDU (1).

Data from a smaller cohort (162) has also been analyzed for disease progression and cause of death in those found deceased. Forty-seven (29%) were found to be deceased, giving a 5 year survival rate of 80% following HIV infection. All subjects found alive had not received antiviral therapy even though 18% reported complications attributable to HIV. Median CD4 counts in this group was 286/mm with 37% below 200. CD4 counts were found to be inversely correlated to HIV RNA concentration.

1c. 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 produced 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. However, 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 clinical 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 responses 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. Accomplishments:

a. Enrollment:

HIV Seropositive = 98 (49 male, 49 female)
HIV Seronegative = 67 (37 male, 30 female)

b. Serology: HIV serology on the 98 seropositive has been completed, 9/98 were found to be subtype E and 8/98 were found to be subtype B.

c. HIV subtypes determined: E - 90; B - 8

d. NK functional studies conducted on 40 HIV-neg and 17 HIV-pos subjects

e. Viral load quantitation in mucosal compartments begun.

Plans:

a. Enroll volunteers from vaccine trials to assess induced immune responses in mucosal compartments.
b. Establish baseline viral load levels and HIV-specific IgA and IgG from various compartments of seropositive subjects.
c. Determine compartment-specific viral load changes in subjects with more than 5 visits.

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.

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

This study was completed. Manuscript is in preparation.

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

This study was completed. Manuscript is submitted for publication.

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

1. 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.

2. 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.

3. 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.

4. Accomplishment

Prevalence: One thousand and two women were enrolled. 39 (3.9%) were found to be HIV positive at the time of enrollment. Factors that were associated with HIV-1 prevalent infection included the following: Age, younger women were at greater risk; Length of the current marriage, the shorter the length of the marriage the greater the risk; History of syphilis, history of ulcerative STD, history of using antibiotics for prophylaxis of STD, tattooing in this population of women, and the number of sexual partners.

Recruiting Success: One-thousand-three hundred and seventeen women were interviewed, of these 1172 were eligible. Those eligible were asked if they were interested in participating, over 85% agreed to enroll. There was probably some self-selection in those that made themselves available for interviewing. Enrollment was conducted from the end of February to early June (3.5 months) a shorter time than was anticipated. An estimate was made of the proportion of the clinic population that enrolled in the study. For the three health centers approximately 25% of the clinic population was enrolled in the study.

Follow-up Success: Follow-up rates were determined for the 6-month visit, the 12-month visit and for those that were seen at both visits. 92% were seen at 6 months, 90 % were seen at 12 months, and 89% were seen at both visits. The follow-up rate decreased as the age decreased. The youngest age group having the worst follow-up rate- only 84% were seen at both visits. Looking specifically at dropouts, 35 persons were lost to follow-up because they moved, 22 were unable to be contacted, one died, and one was in prison. At the final visit volunteers were asked what would encourage them to participate in a vaccine trial. The answers included monetary compensation, health insurance, convenient appointments, recognition from family and friends, helping Thai society, reimbursement for travel, and a complete physical exam. Asked to indicate the one thing that would be most important, they ranked 1. a complete physical exam at the beginning of the study; 2. helping Thai society; 3. health insurance, and 4. monetary compensation.

Incidence: Two women sero-converted during the study, one at the 6-month visit and one at the 12-month visit. There were 889 person-years of follow-up for a rate of 0.22/100 person-years. Both of the incident cases were in women under the age of 30. Volunteers under the age of 30 contributed 334 person-years. Thus the rate for women under the age of 30 was 0.59/100 person-years. The women were both young (22 and 25) while the mean age of the cohort was 32. They had been married a very short time. They had a history of genital blisters or herpes. There sexual experience was limited, one had only had relations with her husband. The other, her husband and one other individual. Unfortunately one was known positive for HIV.

Vaccine Trial Participation: Asked about what things would be a concern in participating in a vaccine trial the most frequent responses were, that they might be given a placebo instead of the vaccine, the possible long term side effects, getting sick sooner if they were to get AIDS, and the concern about false positive test results following immunization. Asked if they would participate in a vaccine trial 37% stated that they would definitely participate, 19% said they would very likely participate. Thus 56% said that they would either definitely or very likely participate in a vaccine trial.

Summary: Enrollment was higher than expected, attendees at family planning clinics are very willing to participate in studies such as these. Prevalence rates were much higher in the lower age groups and the recently married. Future studies will target this younger population. There was willingness to receive the hepatitis B vaccine and good compliance with the program. Thus the family planning clinics provide a well-defined and existent infrastructure for recruitment and enrollment. The excellent follow-up rate demonstrates the value of family planning clinics for these and other long-term prospective studies.

Plans:

The prevalence figures and the two incidence cases suggested what should be obvious that the risk is in the younger population. The plan at the present time is to follow another 1000 women for 12 months. These women will all be under the age of 30. Each of the original health centers will continue to follow those women that were in this age group at the time of entrance into the original study. They will enroll additional women in this age group from their clinic populations. In an effort to see how expandable this cohort is, three additional health centers will screen and refer potential volunteers to the original 3 health centers. This effort began early September and there are presently over 600 women enrolled.

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

1. 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.

2. 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.

3. 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.

4. Accomplishments:

Enrollment: Enrollment started in March and was completed in June. The first 6-month follow-up began in September. In Chong Samaesan approximately 25% of the population in the age range from 20-40 was enrolled. There is greater representation by females than males, and in the 25-29 year old age group over 50% of the age group is enrolled in the study. In Phlutaluang the proportion of the population in the study is considerably lower only 13%.

Prevalence: Five percent of the 1500 persons enrolled were HIV positive at the time of enrollment. The rate from Samaesan was 7% and that at Phlutaluang 3.8%. There was a suggestion that younger persons were at greater risk of being HIV positive, 76% of the sero-positive females were under the age of 30 while only 59% of the sero-negative females were under that age. In males the trend was the same although not as dramatic, with 71% of the sero-positive males were under the age of 30, but only 62% of the sero-negatives were less than 30.

Years of marriage also showed a difference between the sero-positives and the sero-negatives, 72% of the sero-positive females had been married less than 5 years, while only 30% of the sero-negative females had been married for that short a period of time. The same was seen among the males, 79% of the sero-positive males had been married for less than 5 years, only 51% of the sero-negative males had been married for a similar short period of time.

There was very little difference between the groups with respect to education. Perhaps male positives were slightly less educated than the sero-negative males, 65% of the positives completing 6 years or less, while only 42% of the sero-negatives had this minimal level of schooling.

As anticipated fishery workers contributed a number of the prevalent cases among the males. However, only 78 people reported working in the fishery industry. 26% of the males working in the fishery industry were HIV positive at the time of enrollment.

There was a striking difference in injecting drug use between the sero-positive males and the sero-negative males, 39% of the sero-positives compared to 2% of the negatives gave a history of previous drug use. With oral recreational drug use again there was a suggestion that sero-positive males were much more likely to have used oral drugs than sero-negative males. Among the females there was no difference.

When asked if they considered themselves at risk, the prevalent cases were more likely to indicate that they were at high or moderate risk than the sero-negative individuals.

When asked about their husband/steady partners sexual experience, although there was no difference between the sero-positive and sero-negative the number of women who said that their partner had other sex partners was very high. Asked about condom use with their partner, as expected very few ever use condoms with their steady partner.

A large number of both the sero-positives and the sero-negatives had a friend that had died of AIDS. Approximately 50% of the cohort had a friend or family member die of AIDS.

Summary: Enrollment of the cohort using the health centers has been better than anticipated. The prevalence rate suggests that there should be a reasonable incidence (but that has not been the experience in other cohorts). If the incidence is at a level that would make a phase III trial practical then the ease of expandability of this cohort would make it a prime candidate for a phase III trial.

Plans:

The plan is to expand the study from 1500 to 2500 persons, increasing the confidence about the incidence that is observed. Two additional health centers will be incorporated into the study, Taothan, and Bang Sare, both in the Sattahip District. The age range of the participants will be limited to those under the age of 30. Two models will be evaluated. In one model the health center will solicit, screen, enroll, and follow-up the volunteers, only lab work will be

centralized. In the other model a health center will solicit and screen potential volunteers and then refer them to a health center that will enroll and follow them.

Taothan began enrolling volunteers in July and in less than 8 weeks had enrolled 500 volunteers under the age of 30. The prevalence rate in that group was 4.4%. Bang Sare began enrollment the middle of September and should be completed by the end of October.

3. HIV-1 Vaccine Testing

a. Screening and evaluation of potential volunteers

1. 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.

2. 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. The screening protocol has been modified to include EBV transformation. The ability to get transformed cell from volunteers is required for CTL assay in the prime - boost protocol.

3. Results

Screening the volunteer for the prime-boost studies is planned to start in FY2000.

b. Phase I trial of Biocine HIV SF2 gp120/MF59 vaccine

Study was completed. Manuscript is accepted for pulication in "Vaccine".

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

1. 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.

2. 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.

3. 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.

4. Results

This study is completed in July 1999. There was no serious adverse event related to the vaccine reported. There was no breakthrough infection. The preliminary data suggested that these vaccines are safe. This immunogenicity data is under analysis.

d. A phase I/II Trial of Pasteur Mérieux Connaught (PMC) live Recombinant ALVAC-HIV (vCP1521) Prime With VaxGen gp120 B/E (AIDSVAX™ B/E) Boost in Thai HIV-1 Seronegative Adults

1. Introduction

HIV-1 exists as multiple genetic subtypes (currently denominated A-I, O). In the United States, the vast majority of HIV-1 strains are subtype B. However, in Thailand the majority of new infections are with subtype E.[3] Data from antibody cross-reactivity studies demonstrate that binding and neutralizing antibodies from subtype B and E infected subjects react preferentially with viruses from the same subtype.[4,5] These studies illustrate the technical limitations of the formal approach to the development of HIV-1 vaccines, viz., the genetic and antigenic variability of HIV-1, the lack of specific knowledge of immune correlates of protection, and the

lack of a predictive HIV-1 animal model.[4,6,7] In this setting a historically valid approach would be to empirically identify and test products that elicit strong humoral and cellular immune responses to viral strains prevalent within the region where the candidate vaccine will be evaluated. The approach defined within this protocol incorporates genes/proteins isolated from individuals infected with Thai genotype E. Years of effort, by many people, have finally resulted in the availability of clade E immunogens for clinical evaluation as a candidate HIV vaccine.

HIV Vaccine Development in Thailand

In response to this epidemic, the Royal Thai Government has developed and implemented a comprehensive plan for prevention and control of HIV, and preventive HIV vaccines are considered an integral component of this plan.[8] Consequently, the National AIDS Committee of Thailand established a Subcommittee for HIV Vaccine Trials with responsibility for coordinating and overseeing efforts in this area.

In 1991, the World Health Organization (WHO) selected Thailand as a site for evaluation of candidate HIV vaccines.[9] Since then the WHO has provided consultation (now through UNAIDS) to the Subcommittee for HIV Vaccine Trials. Through multiple partnerships and collaborations, Thailand has actively carried out its National Plan. Three trials of preventive HIV vaccines have been completed, and two others were fully enrolled in 1998.[10] The combination of planning, collaboration and commitment to HIV vaccine development has put Thailand in a position of international leadership concerning HIV vaccine development.

The Thai AIDS Vaccine Evaluation Group (TAVEG) is made up of clinicians and scientists from the Royal Thai Army, Mahidol and Chiang Mai Universities and the U.S. Army.[11] In collaboration with Chiron Vaccines, the TAVEG has completed one phase I/II HIV vaccine trial and is carrying out a second.[12,13] The latter is an evaluation of a vaccine construct designed specifically for the HIV subtypes (E and B) most prevalent in Thailand. Through this new protocol, the TAVEG expands its collaboration to include products from Pasteur Mérieux Connaught and VaxGen, Inc in this prime-boost protocol proposal.

HIV-1 Envelope Vaccine Candidates

A variety of HIV-1 envelope (Env) vaccine candidates have been tested in both seronegative and seropositive populations. Studies of recombinant envelope gylcoprotein vaccines expressed in Chinese Hamster Ovary (CHO) cells have demonstrated safety and immunogenicity. To date, efficacy of this approach in humans remains unevaluated.

Studies of AIDSVAX™ B/E (VaxGen) in rabbits demonstrate that the product is highly immunogenic with binding antibody titers in excess of 1:100,000 after the second vaccination. These antibodies appear to neutralize homologous isolates in a PBMC neutralization assay (titers 1:20 to 1:1000). Using a similar vaccine, AIDSVAX™ IIIB, two chimpanzees immunized at 0,1,8 months were protected from 10 times the CID50 or 40 times the TCID50 of HIV_{IIIB} at 3 weeks post 3rd vaccination. Similarly, 3/3 chimpanzees vaccinated with AIDSVAX™ MN and challenged with 20 CID50 or 748 TCID50 of HIV_{SF2} were protected from infection. A phase III

clinical trial of AIDSVAX™ B was initiated in the U.S. and is planned in Thailand; data are pending. Data from a phase I trial of AIDSVAX™ E in Thailand are being collected and analyzed.

Canarypox HIV (ALVAC-HIV) Vaccine Candidate

The PMC ALVAC-HIV vaccine (vCP1521) is a preparation of recombinant canarypox virus expressing the products of the HIV-1 *env*, *gag,* and *pro* genes. The genes are inserted into the canarypox C6 locus under the control of the vaccinia virus H6 and I3L promoters, respectively. The gp120 *env* sequence is derived from the subtype E HIV-TH023 strain, but the anchoring part of gp41, Gag, and Pro are derived from HIV-LAI. Co-expression of Gag and Env, and appropriate Gag processing by protease, in a vaccinia expression system, results in the formation of virus-like-particles that bud from the cell membrane.[25] Thus, ALVAC-infected cells likely present Env and Gag proteins in a near-native conformation. In addition, intracellular processing of foreign HIV-1 proteins via the MHC class I pathway facilitates stimulation of cytotoxic T-lymphocytes. Part of the rational for use of a subtype B gag in the clade E ALVAC product is that portions of the gag gene are conserved among virus subtypes. Therefore, Gag CTL elicited by vCP1521 should react with CTL epitopes on subtype E and B viruses. Recent data from an AVEG sponsored prime-boost trial (vCP205 alone or boosted with Chiron SF2 gp120/MF59) showed that $CD8^+$ Gag CTL from some vaccine recipients recognized target cells infected with non-subtype B viruses, including subtype E.[26] The proportion of subjects with cross-reactive CTL and the optimal immunization regimen remain to be determined. The vCP1521 construct incorporates the most conserved viral element (Gag) with a clade-matched Thai genotype E env. Studies have demonstrated good expression of the clade E env element from vCP1521 infected human cells. (J. Cox, unpublished data). Moreover, vCP1521 primed effectively for a subsequent Thai gp boost (0.5-0.9 log increase in GMT binding antibody to Thai E gp160; F. Boudet, unpublished data). Extensive testing of ALVAC candidate vaccines encoding a variety of different viral products have provided no evidence of toxicity in mammals.[27] The relevance of canarypox antibody to subsequent ALVAC injections is unknown and is the subject of study in the ongoing AVEG022 (no data available). In general, anti-canarypox antibody titers are stable after the second injection; moreover, there are progressive improvements in immunogenicity of the vaccine with subsequent injections. In addition, AVEG012 showed equivalent frequency and magnitude of HIV specific responses in vaccinia naive and immune individuals.[28]

Rationale for immune priming with a live vectored vaccine (Prime-Boost strategy)

While gp120 antigens elicit a strong humoral response, they have usually not induced an anti-HIV $CD8^+$ specific CTL response. In contrast, live recombinant vaccinia virus constructs encoding HIV-1 genes can infect mammalian cells causing them to express HIV-1 proteins.[29] Since vaccinia virus can rarely cause a severe disseminated vaccinia syndrome in immunocompromised subjects,[30] Pasteur Mérieux Connaught (PMC) has developed a recombinant canarypox virus (ALVAC) that can express foreign genes. In mammalian cells, canarypox undergoes an abortive cycle of replication and does not produce infectious progeny.[27,31] This suggests that ALVAC will not disseminate or be transmitted to unvaccinated contacts.

PMC has utilized the ALVAC construct to express genes from rabies, measles, cytomegalovirus and Japanese encephalitis virus, and clinical trials with these constructs have demonstrated safety and immunogenicity. No serious adverse events attributable to the ALVAC vaccine have been reported in over 800 subjects receiving an ALVAC construct (PMC unpublished data).[27] Recombinant canarypox constructs elicit moderate to strong $CD8^+$ restricted CTL in human volunteers, presumably due to antigen processing via the MHC class I pathway.[32,33] Although ALVAC constructs generally elicit both antibody and CTL responses, the level of antibody can be significantly boosted by administration of a soluble protein antigen. In both the guinea pig and macaque models reported above, gp160MN/LAI-2 significantly boosted antibody responses primed with ALVAC-HIV. Thus, one approach to inducing a strong humoral and cellular immune response is priming with an ALVAC construct and boosting with the appropriate soluble protein antigen.

2. Study Objectives:

This Phase I/II is a comparative trial of the safety and immunogenicity of ALVAC-HIV (vCP1521) priming with two different doses of a bivalent AIDSVAX™ B/E gp120 boosting. If immunogenicity criteria are met, Phase IIb will be proposed and initiated separately

3. Methods

PROJECT PHASE: Phase I/II

1) Part A: Phase I (open label) description of the acute safety and tolerability of ALVAC-HIV (vCP1521).

2) Part B: Description of the safety and immunogenicity of ALVAC-HIV (vCP1521) **priming** with either 200 μg or 600 μg (100 or 300 μg each B and E gp120) of the bivalent AIDSVAX™ B/E gp120 **boosting**.

Study Design: A Phase I/II study

Part A:

Group I: ALVAC-HIV (vCP1521; 106.53 CCID50) will be tested separately for acute safety and tolerability in a group (Group I) of 5 low risk, HIV seronegative Thai adults. If there are no vaccine-related grade 3 or above adverse events by 72 hours after immunization#2 in part A, part B will be initiated.

Part B:

Group II: 45 low-risk HIV seronegative Thai adults will be given ALVAC-HIV (vCP1521; $10^{6.53}$ $CCID_{50}$) priming at weeks 0, 4, 12, and 24. At weeks 12 and 24, vCP1521 will be administered with 200 μg of a bivalent AIDSVAX™ B/E gp120 boost (100 μg for each B and E gp120), while 15 other subjects will receive a placebo.

Group III: 45 low-risk HIV seronegative Thai adults will be given ALVAC-HIV (vCP1521; $10^{6.53}$ $CCID_{50}$) priming at weeks 0, 4, 12, and 24. At weeks 12 and 24, vCP1521 will be administered with 600 μg of a bivalent AIDSVAX™ B/E gp120 boost (300 μg for each B and E gp120), while 15 other subjects will receive a placebo.

If defined immunogenicity criteria are met, **one** dosage of HIV-envelope subunit boost will be selected and a Phase IIb (see Appendix 17.6) will be initiated with lots of candidate products manufactured at a 'scale' to support future efficacy trials.

SUBJECTS: As many as 125 healthy, HIV seronegative Thai adults, 20 to 50 years of age, available for at least 1 year or follow-up. There are 60 subjects in each group (45 vaccinees in 1a and 2a, 15 placebo recipients in 1b and 2b).

Product Descriptions:

ALVAC-HIV (vCP1521), produced by Pasteur-Mérieux-Connaught (PMC), is a recombinant canarypox vector vaccine that has been genetically engineered to express subtype E HIV-1 gp120 (92TH023) linked to the transmembrane anchoring portion of gp41 (strain LAI), and HIV-1 gag and protease (LAI strain). vCP1521, batch S3484, is formulated at a dose of $10^{6.53}$ $CCID_{50}$ with 10mM Tris HCl, pH 9 and lactoglutamate.

Placebo-ALVAC, ALVAC carrier, batch S3289, contains 10mM Tris HCl, pH 9 and lactoglutamate without virus.

AIDSVAX™ B/E, produced by VaxGen, is a bivalent HIV gp120 envelope glycoprotein vaccine containing a clade B envelope from the HIV-1 strain MN and a clade E envelope from the HIV-1 strain A244. The recombinant gp120s are produced in a genetically engineered Chinese hamster ovary (CHO) cell lines. The envelope glycoproteins of MN and A244 are coformulated and administered in alum at a dose of either 200 μg (100 μg each) or 600 μg (300 μg each).

AIDSVAX™ B/E placebo - alum

Route Of Administration: Intramuscular[2].

Immunization Schedule:

Part A

			Months			
Group	Vaccine/ Placebo	gp120 Adjuvan	0	1	3	6
I	5/na*	na	vCP1521	vCP1521	vCP1521	vCP1521

Part B

II	45/15[1]	alum	vCP1521	vCP1521	vCP1521+ 200 μg gp120 B/E	vCP1521 + 200 μg gp120 B/E
III	45/15[1]	alum	vCP1521	vCP1521	vCP1521+ 600 μg gp120 B/E	vCP1521 + 600 μg gp120 B/E

*na = not applicable

Study Endpoints:

Safety and Tolerability: Tabulation of adverse effects and laboratory abnormalities: Subjects will be observed for 30 minutes following immunization for evidence of immediate local and systemic reactions. They will be instructed to watch for local and systemic reactions for 7 days post-immunization and will be evaluated by an investigator if significant symptoms are reported. Routine measurements of hematology, serum chemistry, and urinalysis laboratory tests will be performed.

Immunogenicity: Description of the following primary measures of immunogenicity

Humoral:
- •Binding antibodies measured by ELISA to clade E and B vaccine component antigens.
- •Neutralizing antibodies against clade B and E viruses grown in PBMC and transformed cell lines.

Cellular:
- •Lymphocyte proliferative responses to genotype B and E vaccine component antigens.
- •Cytotoxic T-lymphocyte (CTL) responses against clade B and E vaccine component antigens.

Immunogenicity Criteria for Advancement to Phase IIb:

Previous prime-boost studies of ALVAC-HIV and subunit envelope products have generally shown roughly 70-80% of recipients developing neutralizing antibody, 60% possessing significant proliferative responses to immunogen, and 50% developing CTL. Given the number of persons enrolled in groups III and IV, we would expect to find, as a minimum, ≥70% developing NAb, ≥60% having significant LPA, and ≥30% having CTL

Humoral: > 70% recipients develop >50% reduction in p24 to E and B subtypes in an H9 based neutralization assay

Cellular: > 60% of recipients develop an LSI > 5 to HIV-specific vaccine components at 2 timepoints post vaccination

>30% of recipients develop CTL to HIV vaccine component antigens. Positive CTL is defined as lysis ≥ 10% which occurs at a single time point at ≥2 E:T ratios or at multiple time points at any E:T ratio. Cummulative % positive will be calculated as % positive (vaccinees) minus % positive (placebos).

Interim Analysis: Preliminary safety and immunogenicity analysis may be performed after the second and third immunizations. A final analysis will occur after all visits are complete.

4. Results

This protocol is in the process of approval from Thai MOPH.

e. A Phase I/II, Double-blind, Placebo-controlled Study of the Chiron Vaccines HIV Thai E gp 120 /MF59 Vaccine Administered Alone or Combined with the Chiron Vaccines HIV SF2 gp 120 Antigen in Healthy HIV-Seronegitive Thai Adults.

Addendum #1 -August 6, 1999

Two booster injections with a higher dose of Chiron Vaccines HIV Thai E gp120 (200μg) / MF59 Vaccine alone in RV114 volunteers previously immunized with Chiron Vaccines HIV Thai E gpl20 (100μg) / MF59 Vaccine or Thai E gp120 (100μg) + SF2 gp120 (25 or 50 μg)/ MF59 Vaccine.

1. Introduction

The present study (V26/6P1 / RV114) has evaluated the safety and immunogenicity of three doses of Thai E gp120/MF59 vaccine (25, 50, 100 μg) administered alone or in combination with the HIV-1 SF2 gp120 (25, 50 μg) antigen in healthy Thai adult subjects. The data generated so far indicate that products and well tolerated even at the highest cumulative dose (SF2 + E gp120) administered (150μg). However, the preliminary immunogenicity data seem to indicate that V3 antibody responses were relatively weak, and no neutralizing antibody responses against Thai E primary isolates were induced. It is speculated that the doses administered might not be optimal to raise such antibodies to the threshold of detection.

It is therefore proposed to offer two additional booster injections, at three month intervals of the Chiron Vaccines Thai E gp120/MF59 at a higher dose (200μg) with the expectation to enhance the binding and neutralizing antibodies, especially those directed against a Thai E primary isolate.

2. Objectives:

- **Safety Objective:**

  To evaluate the safety of two additional booster doses (given three months apart) of Chiron Vaccines human immunodeficiency virus (HIV) Thai E gp 120 (200 μg) / MF59 vaccine alone in RV114 previously immunized Thai volunteers.

- **Immunogenicity Objective:**

  To evaluate the immunogenicity of the two additional booster doses of the Chiron Vaccines Thai E gp 120 (200 μg) / MF59 vaccine alone in RV114 previously immunized Thai volunteers.

3 Method:

This addendum will be a double-blinded trial. The volunteers as well as the clinical team responsible for the medical evaluation will be blinded for the injection. A person not involved in subject evaluation will prepare the vaccine dose. Twenty-four volunteers already enrolled in RV114 at the AFRIMS and VTC study centers and having received the Chiron Vaccines Thai E gp120 (100 μg) / MF59 vaccine alone or simultaneously with the Chiron Vaccines HIV SF2 gp120 antigen (25 or 50 μg) will receive intramuscularly two doses at a three month interval of Chiron Vaccines Thai E gp120 (200 μg) / MF59 vaccine. Six volunteers having previously received a placebo (at AFRIMS and VTC) will be given two intramuscular injections of MF59.

Volunteers will be enrolled into this addendum and receive their first immunization between 9-18 months after their last injection of gp120 in RV114. Subjects will be followed up for 3 months after the second booster immunization according to the attached schedule of events. In addition, all subjects will be followed for 12 months after the final study visit for HIV testing and will also be contacted annually for a period of 5 years after the last immunization to monitor their general health according to guidelines outlined in the National Plan for AIDS Vaccine Development and Evaluation. Volunteers who experience "breakthrough" HIV-1 infection during the trial, or up to 18 months after the last immunization, will be offered enrollment in study to evaluate the "natural history" of HIV-1.

Number of Subjects: 30 subjects (24 vaccines and 6 placebo recipients).

Subject characteristics and main criteria for inclusion/exclusion: Healthy Thai adult volunteers already enrolled in the protocol RV114 and immunized with Thai E gp120 (100 μg) / MF59 vaccine or Thai E gp120 (100μg) + SF2 gp120 (25 or 50 μg)/ MF59 vaccine or a placebo, 20 through 60 years of age; available for at least six months of follow-up (AFRIMS and VTC study centers ONLY). Subjects who currently engage in or have participated in high-risk behavior for HIV infection within the past 6 months are excluded.

Test vaccine, antigen content, dosage regimen, route of administration, lot number:

1. Chiron Vaccines Thai E gp120 Antigen, containing 0.8 mg/mL dose (0.35 mL vial.) Lot # MGAPL002
2. MF59 Citrate Adjuvant Emulsion, supplied in 0.4 mL per vial. Lot # NECPN001a

Two injection doses of 200 μg in 0.5 mL each, given intramuscularly at 3 month interval.

Measures of safety: All subjects and the clinic personnel will be blinded. Subjects will be observed for 30 minutes following immunization for evidence of immediate local and systemic reactions. They will be instructed to watch for local reactions (ie, pain, erythema, induration and increased injection-site temperature) and systemic reactions (ie, fever, chills, fatigue, headache, nausea, myalgia, arthralgia, rash) for 7 days post-immunization. They will be contacted by the study staff between 24 to 72 hours post-immunization 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 second booster injection will be collected. After that, 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.

Measures of immunogenicity:

- Binding antibodies to Thai E gp120 antigens, measured by enzyme-linked immunosorbent assay (ELISA).
- Neutralizing antibodies to Thai E HIV-1, measured by TCLA or PBMC neutralization assays.
- Cellular T lymphocyte proliferation responses to Thai E gp120 & recall antigens & mitogen.

Interim Analysis:
Preliminary safety and immunogenicity analyses will be performed after the first and second immunizations. A final analysis will occur after all visits are complete.

Criteria for assessing immunogenicity study objectives:
- A twofold difference of Thai E ELISA V3 and gp120 and neutralizing antibody GMTs at peak titer one month post-immunization number 3 versus at one month after the second 200 μg immunization booster will be considered immunologically significant.

Flow Chart Summary

Study visit	A1	A2	A3	A4	A5
Week	0	2	12	16	24
Month	0		13	4	6
Immunization	X		X		

Administrative requirements					
Consent	X				
Clinical requirements					
Physical exam	X	X	X	X	X
Interim history	X	X	X	X	X
Reactogenicity exam	X	X	X	X	X
Extended risk assessment					X
Abbreviated risk assessment	X		X		
Risk behavior counseling	X	X	X	X	X
Clinical Laboratory					
HIV-1 serology (1 mL)	X				X
A-LT, creatinine (3 mL))	X	X	X	X	X
CBC, differential, platelets (3 mL EDTA)	X	X	X	X	X
Urine dipstick for blood/protein	X	X	X	X	X
Urine Pregnancy test	X		X		X
Immunogenicity labs					
Binding and neutralization (5 mL) SS	X	X	X	X	X
T cell Lymphoproliferation 8mL x 3 CPT tubes (24mL)	X		X	X	X
HIV RNA and DNA PCR (from CPT tube above)	X				X
Total blood volume (mL)	**36**	**11**	**35**	**35**	**36**

4. RESULT:

This protocol is in the process of approval from Thai MOPH.

f. 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 Check 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.

During FY99 a CAP inspector from WRAIR provided a technical assist visit which included an inspection of all vaccine trial laboratories. The purpose of the inspection was to determine readiness of the laboratories for a certification inspection in the future and to point out problems and areas that require continued improvement prior to that inspection. The visit was highly successful and while numerous problems were identified none were considered unresolvable. The visiting inspector provided training and assisted in the planning and implementation of continuing improvements in the QA/QC program.

During July the current QA/QC Coordinator was able to participate in CAP Inspector Training in the United States. This will increase the expertise of the Coordinator in assisting the laboratories in continuing improve and achieve certification in the future.

The laboratories continue to participate in several external Proficiency Programs including:

a. Streck laboratories UKNEQAS & Australia for flow cytometry
b. CAP - flow cytometry, serum chemistry, viral load, hematology
c. WRAIR/Thai National HIV serology and viral load PT programs.

Results continue to be excellent overall and only occasional discrepancies have been noted. Similarly, the laboratories continue to participate in the NRL (Australia) HIV Viral Load Program. Again, results of this program have been excellent throughout.

Plans:

Current plans call for the WRAIR CAP inspector to return to provide another assist visit to again assess the readiness of the laboratories and determine the level of improvements. It is hoped that the laboratories will be ready to actually apply International CAP Certification during FY 2000. It is also hoped that additional laboratory staff will be able to obtain CAP inspector training the year.

4. Laboratory

Accomplishments:

1. Diagnostic
   a. Second HIV viral load platform in place at AFRIMS (ROCHE Amplicor v1.5).
   b. Assay established to differentiate HIV subtypes B and E within the gag region using restriction endonucleases.

2. Vaccine Immunogenicity: CTL assay
   a. HIV-specific CTL assays operational at AFRIMS.
   b. HIV-specific CTL assay tech-transferred to Siriraj.
   c. Baseline CTL data collected on HIV-seronegative subjects to HIV subtype E env.

3. Vaccine immunogenicity: Neut. Ab.
   a. Characterized panel of 53 Thai viruses (B' & E) for sensitivity to neut. Ab.
   b. Reverse transcriptase assay established and applied to neutralization assay
   c. Developed flow cytometry assay for neutralization of clinical isolates (subtypes A-G).

4. Viral surveillance: potential B/E recombinant viruses observed in cases with E serotype/B genotype and dual serotypes.

Plans:

1. Cellular immunology
   a. Major effort will be performing CTL assays for phase II prime-boost vaccine trials.
   b. Determine frequency of vaccine-induced CD4 precursors.
   c. Develop ADCC assay and assess role in Thai infections.

2. Humoral immunology
   a. Continue development of HIV subtype E panel for neutralization assay, both primary and TCLA viruses.
   b. Provide reagent panels to collaborating labs.
   c. Assess Ab responses in Thai specimens with differential rates of disease progression.

3. Virology
   a. Confirm/refute presence of recombinant viruses by sequencing of full length genomes from B/E discordant cases.
   b. Determine virologic characteristics associated with sensitivity to neutralization.

4. Diagnostics
   a. Employ genetic (RNA) assays as part of diagnostic algorithm for detection of HIV infection among vaccinees in prime-boost trial.

5. *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	1998	1999
Central*	1.2	2.2	2.9	3.0	2.6	2.5	2.0	1.8	1.4	1.3
Bangkok	1.2	2.8	3.3	3.2	2.9	2.6	1.7	2.2	2.2	3.0
Northeast	0.9	1.8	2.4	2.6	2.6	1.6	1.6	1.1	1.1	0.7
North	6.1	6.5	7.5	7.3	5.0	3.4	3.3	2.5	1.8	1.3
South	1.6	2.2	2.6	2.8	2.2	2.0	2.1	2.6	2.0	2.2
Total	**1.9**	**2.9**	**3.5**	**3.7**	**3.0**	**2.4**	**2.0**	**1.9**	**1.6**	**1.6**

* 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 7different species, including three non-human primate species and four rodent species.

D. Laboratory Science Support

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.

During FY99, the glassware section received approximatly 50,000 items of glassware for cleaning and decontamination. They distributed approximatly 36,000 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.

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. It provides knowledge of disease course in Thais by enrolling new incident cases (via cohort studies) and contacting old patients for return visits to increase numbers with long F/U periods. We plan to explore for occurrence of recombinant viruses in Thailand utilizing discordant serotype vs. genotype cases. The difference between disease progressors and non-progressors in the Thai population will be explored.

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 and the community based cohort in Sattahip are on-going in Rayong and Chonburi provinces. Enrollment was excellent.

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 accepted for publication. A large phase I/II trial of a subtype E HIV subunit vaccine (rgp120) was completed. Data regarding safety and immmunogenicity is under analysis. There was no serious adverse events related to the vaccine. Two of the prime-boost protocol are on going.

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.

IV. Abstracts and Publications (FY1999)

Reference Type: Conference Proceedings
Record Number: 84
Author: Nitayaphan, S; Brown, AE
Year: 1999
Title: HIV in Asia Pacific Militaries, results of survey of APMMC VIII Participants
Conference Name: APMMC IX
Conference Location: Bangkok, Thailand
Abstract: Throughout history, militaries have suffered from sexually-transmitted diseases among the troops. The most lethal of these disease risks is infection with the human immunodeficiency virus (HIV). In some regions of the world, HIV infection is considered to be a serious problem in both military as civilian sectors of society. At the same time, militaries can provide effective leadership in a nation's flight against this pandemic.
To assess the HIV threat and response to the militaries participating in the VIIIth Asia Pacific Military Medicine Conference (APMMC), a questinaire was sent to 1998 participate countries. Major areas covered by the questionaire include: HIV epidemiology within nation and military, military policy and practice in response to the HIV epidemic, and attitudes toward HIV vaccines. Results gathered through this survey will be presented in summary form, maintaining, natinal anonymity. This initial step in networking among the APMMC participants will allow the medical departments of these diverse militaries to learn from each other and encourange further networking in the future.

Reference Type: Conference Proceedings
Record Number: 102
Author: Chiu, J; Eiumtrakul, S; Rangsin, R; Sirisopana, N; Eamsila, C; Khamboonruang, C; Markowitz, L
Year: 1999
Title: Morbidity and Mortality offhal Men 5 to 7 years after HIV-1 Scroconversion
Conference Name: Fifth International Congress on AIDS in Asia and the Pacific
Conference Location: Kuala Lumpur, Malaysia
Abstract: **Objective**: To describe clinical outcomes in a cohort of Thai men 5 to 7 years after HIV- 1 infection.
Methods: An ongoing cross-sectional survey of a cohort of former Royal Thai Army (RTA) recruits who seroconverted between 1991 to 1994 while based in Rang-kokand 6 Northern provinces. To maintain anonymity. at least (1 former RTA recruit known to be seronegativc at time of discharge and from the some district as each seroconvener was included in the survey. All subjects were contacted in a blinded manner regarding their HIV status. Survival was deterniined using Kaplan Meier. CD4 count was determined by flow cytometry and viral load was quantitated using the Roche Amplicor (version 1.5) assay.
Results: As of May 1, 1999, surveys have been conducied In 4 provinces involving 162 HIV- 1 Infected subjects. Of these 104 (64%) were known to be alive, 47 (29%) were known deceased and 11 (71%) could not (be traced. (In contrast 4% (6 of 151) of seronegative controls were

found dead.) Five-year survival following HIV-1 scroconversion was 80 %. Cause of death could be ascertained for 41 subjects of which 34 (72%) deaths were directly attributed to HIV. Median Time from seroconversion to HIV attributable death was 252 weeks (range, 87-356 wk). All subjects known to be alive have not received antiretroviral therapy even though 18% reported HIV- associated complications such as chronic fever, weight loss, diarrhea and tuberculosis. HIV-associated findings such as thrush, eachexia were seen in 29 of 74 (39%) subjects who agreed to complete physical examination. HIV RNA was detected in plasma from 72 of 74 subjects who agreed to participate in blood collection (median. 4.7 log RNA copies/mL). Median CD4 count was 296/mm with 37% below 200/mm. There was no correlation in the distribution or CD4 counts and viral load by fime since seroconversion. However, there was an inverse correlation between HIV RNA concentration and absolute CD4 count (r=41. p=0.01).

Conclusions: This report provides the first data on mortality among Thai adults with a known duration of 141V infection. 64% survived 5-7 yerars after seroconversion. Though the 5-years survival for this cohort is similar to that mponcd from lite West. nearly all surviving subjects tested have detectable HIV RNA in plasma and 1/3 have clinical findings or CD4 counts that indicate significant immune compromise.

Reference Type: Conference Proceedings
Record Number: 97
Author: Darden, J; Polonis, V; deSouza, M; Chantakulkij, S; Brown, A; Birx, L; K, Pattanapanyasat.
Year: 1999
Title: Flow Cytometric Quantitation of Intracellular HIV-1 p24 Protein: Application to HIV-1 Subtype E Neutralization
Conference Name: Fifth International Congress on AIDS in Asia and the Pacific
Conference Location: Kuala Lumpur, Malaysia
Abstract: **Background:** HIV-1 subtypes E and B have been shown to be the circulating subtypes in Thailand and several other countries in Southeast Asia. Clinical trials testing candidate vaccines from both HIV-1 subtypes B and E have required further development of immunogenicity assays to detect responses to antigens of both subtypes. The variability in results seen with current p24 neutralization (NT) endpoints prompted us to assess the utility of flow cytometry for monitoring the neutralization of HIV clinical isolates.
Methods: A modified NT assay employing CD8-depleted peripheral blood mononuclear cells was performed; cells harvested at day 4 were fixed, permeabilized, stained with a directly conjugated anti-HIV p24 monoclonal antibody, and analyzed by flow cytometry to quantitate infected CD4+ cells. A panel of HIV-1 subtype B and E clinical isolates was tested against pooled patient plasma with characterized V3 loop peptide serotypes.
Results: Strong subtype-specific neutralization of clade E viruses was observed using the E V3 serotype plasma pool. Some cross-neutralization of one E virus using the B V3 serotype plasma pool was also observed and neutralization ranged from 57% to 98% reduction of infected cells. Using uninfected cells from the same culture timepoints, no background staining of uninfected cells was observed.

Conclusions: These data demonstrate that flow cytometric quantitation of intracellular HIV-1 p24 can be used as an endpoint assay to assess clinical isolate neutralization of HIV-1 subtypes B and E. This flow cytometric method has the advantage of being an enumerative assay for quantitating infected cell subsets in cultures at an early timepoint and eliminates the need for cell washing in NT assays. This assay may detect neutralization of isolates from multiple genetic subtypes of HIV-1.

Reference Type: Conference Proceedings
Record Number: 94
Author: de Souza, M; Polonis, V; Khannapho, C; Viputtikul, K; Nitayaphan, S; Birx, D; Brown, A
Year: 1999
Title: Tissue culture adapation of HIV subtype E primary isolates
Conference Name: Fifth International Congrss on AIDS in Asia and the Pacific
Conference Location: Kuala Lumpur, Malaysia
Abstract: **Background:** Current HIV vaccine trials in Thailand employ subunit vaccine candidates derived from HIV subtype E. Neutralisation of HIV is an immunogenicity endpoint in HIV vaccine trials. Current neutralisation assays of subtype E employ peripheral blood mononuclear cells (PBMC) and are prone to inter-donor variability. Adaptation of subtype E to continuous cell lines should reduce inter-assay variability in cell-based neutralisation assays.
Methods: Two HIV subtype E isolates (NP 1255 and NP 1701) were obtained from subjects enrolled in a natural history study in Thailand, with CD4 counts of 247/ul and 531/ul, respectively. Supernates from primary cultures expressing maximal p24 were inoculated into PBMC for virus expansion, and phenotyped using the MT-2 assay. One virus was of the syncytium inducing (SI) phenotype and the other non-syncytium inducing (NSI). Eight days later, PBMC supernates were tested for p24. Infected PBMC were treated with mitomycin C and co-cultured with H-9 cells, and cultures monitored weekly for p24.
Results: Both H-9/PBMC co-cultures expressed p24 over a 4-month period. Supernates were harvested, filtered and passaged into fresh H-9 cells, and monitored for virus production and syncytia formation. Virus was again produced in both cultures as assessed by p24 and reverse transcriptase assays. A second passage in H-9 cells of both viruses was again successful. The V-3 loop for both H-9 adapted viruses was sequenced and compared to the respective V3 parental sequence obtained from PBMC. NP1255 and NP1701 parental sequences showed 7/35 and 6/35 amino acid changes in the V3 region compared to the consensus sequence.
Conclusions: Primary isolates of HIV subtype E of both SI and NSI phenotypes can be adapted to tissue culture and may facilitate in vitro neutralisation assays using sera from subjects vaccinated with subtype E viruses.

Reference Type: Conference Proceedings
Record Number: 91
Author: Polonis, V; Darden, J; de Souza, M; Gartner, S; Sutthent, R; Pattanapanyasat, K; Birx, D; Brown, A
Year: 1999

Title: Processing of subtype B and E human immunodificiency virus (HIV) proteins in macrophages and T lymphoctes
Conference Name: XIth International congress of Virology
Conference Location: Sydney, Australia
Abstract: In vivo, both T cells and macrophages are infected by HIV. We have studied the biosynthesis and processing of native HIV-1 proteins to assess host cell-specific difference in the life cycle of HIV. Viruses that replicate in both macrophages and T cells were used; the initial studies have been done using HIV-1 subtype B. While the HIV-1 Gag structural proteins appear to be similarly processed in different cell types, we find distinct differences in the intracellular transport and processing of the envelope (Env), as well as the Nef regulartory protein, in specfic cell lineages. In macrophase, intracellular accumulation of uncleaved env gp160 precursor was observed; virion associated env protein was also found to be structurallydifferent in T cells versus macrophages. Currently, vaccine trials are underway to test Env products form HIV-1 subtype B (prevalent in N. America and Europe) and subtype E (prevalent in Southeast Asia). An understanding of teh biogenesis of HIV proteins in human cells will aid in our dissection of relevent immune responses to these antigens. In vitro biosynthesis studies using HIV-1 subtypes B (BaL) and E (F36-CVL) will be presented. Difference int he life cycle of HIV in functionally distinct host cells may have an impact on HIV pathogenesis and on vaccine studies.

Reference Type: Conference Proceedings
Record Number: 90
Author: Leelamanit, W; Leutrakul, T; Jiratchariyakul, W; Ponglikitmongkol, M; Polonis, VR; Panyim, S
Year: 1999
Title: Anti-Tumor and Anti-HIV proteins extracted from momordica charantia cultivated in Thailand
Conference Name: Joint International Tropical Medicine Meeting
Conference Location: Bangkok, Thailand
Abstract: Momordica charantia was reported to be an important source of anti-tumor and anti-HIV compunds. So far, at least three anti-tumor anti-HIV proteins were identified from this plant species including Map 30 (Momordica Anti-HIV protein, 30 kDa) and a- and P-momorcharins. Here, we report the isolation and characterization of anti-HIV proteins from the seeds of two local Momordica charantia species, Mara Khee Nok and long-shaped Chinese bitter melon, cultivated in Thailand. Ammonium salt precipitation and ion-exchange chromatography were used to purify two basic proteins of pl 9-10 displaying molecular weights of 30 kDa. The dialyzed 0-30% and 30-60% fractions of both plants possessed anti-HIV activity at 0. 175 ug/ml. Tested with different tumor cell lines, the anti-tumor activity was seen in dialyzed 30-60% fractions of both species. Interestingly, the fractions also displayed topoisomerase 11 activity by decatenating kinetoplast DNA. The authentic biological activity of purified homogeneous proteins from both plant species needs to be further studied.

Reference Type: Conference Proceedings
Record Number: 98

Author: Karnasuta, C; de Souza, MS; Cox, JH; Polonis, VR; Birx, DL; Brown, AE
Year: 1999
Title: Natural Killer Cell Function in HIV-Seronegative and Seropositive Thais
Conference Name: FIfth International Congress on AIDS in Asia and the Pacific
Conference Location: Kuala Lumpur, Malaysia
Abstract: Background: Natural killer (NK) cells are a discrete subset of lymphocytes. NK cells are operationally defined by their ability to lyse a restricted panel of target cells such as tumour and virally infected cells. It has been reported that NK cell number and phenotype are normal, but functionally defective in HIV-infected patients.
Methods: Thai HIV+ cases were divided into three categories depending on the number of CD4+ lymphocytes (CD4 < 200, CD4 = 200-500 and CD4 > 500 cells/ml). Forty-two HIV seronegative Thais served as controls. NK cells were phenotypically identified using flow cytometry, and calculated as a percentage of total lymphocytes. Functional activity of NK cells was assayed using a chromium release method and was expressed as lytic units (LU). A correlation between CD4 counts and the functional activity of NK cells was determined.

Results: All data shown in the table below, are expressed in arithmetic means with standard deviations.

	Thai HIV-	Thai HIV+ CD4 < 200 Cells	Thai HIV+ CD4 = 200-500	Thai HIV+ CD4 > 500 Cells
NK Cells (% lymphocyte)	16 ± 6	18 ± 8	17 ± 8	15 ± 9
Activity(LU)	24 ± 16	3 ± 8	19 ±11	19 ± 11

We found no significant differences (P > 0.3) in the percentage of NK cells among Thai HIV- and Thai HIV+ groups as had been observed by others. However the LU in Thai HIV-infected subjects was significantly lower (P = 0.004), only in patients with low CD4 counts (CD4 < 200 cells/ml), than that in Thai uninfected subjects.

Conclusions: This study suggests that the activity of NK cells is independent of NK cell proportions but may be correlated to CD4 absolute number during the various stages of HIV infection. NK cells may play a crucial role in the protection against HIV infection; further investigations are still required.

Reference Type: Conference Proceedings
Record Number: 93
Author: Caudle, LC; Pumratana, K; Sirisopana, N; Benenson, M; Tontichaivanich, C; Tungsakul, V; Santativongchai, S; Theppatiphat, P
Year: 1999
Title: Cohort Development for Future Phase III HIV-1 Vaccine Trials Using Women Attending Family Planning Clinics in Rayong Province, Thailand

Conference Name: Fifth International Congress on AIDS in Asia and the Pacific
Conference Location: Kuala Lumpur, Malaysia
Abstract: Objective: To determine the feasibility of establishing a cohort for HIV vaccine efficacy trials among women attending family planning clinics in Rayong Province, Thailand. Initial baseline prevalence data and subtype analysis of HIV-1 isolates, as well as baseline and 6 month F/U behavioral risk data is presented.
Methods: Subjects attending family planning clinics at three sites in Rayong Province, Thailand (Pae, Tapong, and Nernprah) were screened to determine eligibility and willingness to participate in a one year study of HIV-1 incidence. Subjects who enrolled were counseled, had blood drawn for syphilis, hepatitis B, and HIV, and had demographic, general medical, and behavioral risk factor data collected.
Results: Between February and June 1998, 85.5% (1,002/1,172) of eligible women agreed to participate and were enrolled in the study. The mean age was 31.9 years, 99.7% were married and 95.6% planned to remain in the Rayong area for at least 5 years. Sixty-four (64%) of the volunteers felt they were at some risk of HIV disease. There were 39 prevalent cases (3.9%). Seven percent of those less than 30 years of age were positive while only 2% of those 30 years or over were positive. More of the prevalent cases reported having multiple sexual partners (67% vs. 31%) and appropriately saw themselves at high risk of acquiring HIV (15% vs. 5%). There was a significant number of serotype B HIV-1 in this group although the heterosexual epidemic in Thailand is largely serotype E disease. Six month F/U in this group was 92%.
Conclusion: The excellent follow-up rate to date demonstrates the value of using family planning clinic attendees for long-term prospective studies. Their effectiveness for an HIV-1 vaccine trial will depend on the incidence rate and willingness to participate in vaccine trials. With declining HIV-1 disease rates in Thailand and thus larger cohorts needed for these trials, there is a need to develop a cohort in a population that can be expanded in a well established infrastructure.

Reference Type: Conference Proceedings
Record Number: 92
Author: Sirisopana, N; Caudle, L; Tontichaivanich, C; Tungsakul, V; Pumratana, K; Santativongchai, S; Benenson, M; Yomchinda, W; Chaiyaruk, M; Jarerut, S; Nitayaphan, S; Brown, A
Year: 1999
Title: HIV-1 Prevalence in Women attending Family Planning clinics in Rayong Province, Thailand
Conference Name: National AIDS Meeting
Conference Location: Bangkok, Thailand
Abstract: Objective: To determine the feasibility of establishing a cohort for HIV vaccine efficacy trials among women attending family planning clinics in Rayong Province, Thailand. Rates of follow-up as well as subtype analysis of HIV- I isolates were also deemed to be important goals. (Initial baseline prevalence data is presented, the study is ongoing.)
Methods: Subjects attending family planning clinics at three sites in Rayong Province, Thailand (Pae, Tapong, and Nernprah) were screened to determine eligibility and willingness to participate in a one year study of HIV- I incidence. Subjects who enrolled were counseled, had blood drawn

for syphilis, hepatitis B, and HIV as well as demographic, general medical, and perceived behavioral risk factor data collected.
Results: Between February and June 1998, 85.5% (1,002/1,172) of eligible women agreed to participate and were enrolled in the study. The mean age was 31.9 years, 99.7% were married and 95.6% planned to remain in the Rayong area for at least 5 years. Sixty-four percent of the volunteers felt they were at some risk of HIV disease. The follow-up rate for the 6-month visit is currently 92%. (Last day of 6-month F/U visit is 23 April 1999.)
There were 39 prevalent cases (3.9%). These cases were more likely to be younger. Seven percent of those less than 30 years of age were positive while only 2% of those 30 years or over were positive. The prevalent cases also tended to be married for less than 5 years (74% vs. 27%); have fewer than 2 children (64% vs. 39%); and be unskilled or temporary workers. More of the prevalent cases reported having multiple sexual partners (67% vs. 3 1 %) and appropriately saw themselves at high risk of acquiring HIV (15% vs. 5%). Fifty percent of the volunteers had no serological evidence of previous hepatitis B infection. Fifty-six (5.6%) of them had evidence of chronic hepatitis B infection. Sixty-nine percent of the volunteers eligible to receive the hepatitis B immunization series have completed it to-date.
Conclusions: The excellent follow-up rate to date demonstrates the value of using family planning clinic attendees for long-term prospective studies. Their effectiveness for an HIV- I vaccine trial will depend on the incident rate and willingness to participate in vaccine trials. As national HIV prevention programs in Thailand have lowered the risk of HIV infection in traditional high risk populations, a cohort for a phase III efficacy trial will have to be larger than previously planned, hence a need to develop a cohort in a population that can be expanded in a well-defined and established infrastructure, such as MOPH's country-wide family plannine clinics.

Reference Type: Conference Proceedings
Record Number: 89
Author: Brown, AE
Year: 1999
Title: HIV Vaccine testing in Thailand: The Prime-Boost Strategy
Conference Name: Joint International Tropical Medicine Meeting
Conference Location: Bangkok, Thailand
Abstract: Thailand is a world leader in the clinical development of vaccines for the prevention of HIV. The Thai AIDS Vaccine Evaluation Group (TAVEG), in collaboration with the U.S. Army and corporate partners, is testing vaccines specifically designed for the HIV epidemic which threatens Southeast Asia. Phase I & 11 trials of gp 120 vaccines from Chiron are evaluating candidates designed to induce neutralizing antibodies (NA). Complementary to this development of recombinant subunit HIV vaccines, the TAVEG proposes to evaluate a "prime-boost" combination of vaccine candidates designed to stimulate both NA and cytotoxic T-lymphocyte (CTL) immune responses. The "prime" will be a live recombinant canarypox virus (ALVAC strain) and the "boost" will be one of three recombinant subunit proteins. ALVAC-HIV (vCP1521), produced by Pasteur-Meneux-Connaught (PMQ, contains multiple HIV genes: env, gag and protease. The gp120 sequence of the env gene is derived from a Thai subtype E virus. The other, more conserved, genes are derived from subtype B viruses. The

soluble proteins used to "boost" the immune response include those derived from subtype E envelope proteins: gp 120 (Chiron Vaccines), gp 160 (PMC), gp 120 (VaxGen).
The testing of the Thai E HIV prime-boost vaccine candidates will be carried out at the four TAVEG sites: AFRIMS, Siriraj Hospital, and the Faculty of Tropical Medicine (Mahidol University) in Bangkok and RIHES (Chiang Mai University) in Northern Thailand. Based upon the results of NA and CTL assessments, one prime-boost combination will be proposed for efficacy evaluation (phase III trial) as an HIV preventive vaccine in Thai adults.

Reference Type: Conference Proceedings
Record Number: 99
Author: Sukwit, S; Limpairojn, N; Caudle, LC; Sirisopana, N; Tontichaivanich, C; Tungsakul , V; Nitayaphan, S
Year: 1999
Title: HIV-1 Western Blot bamdomg pattern of seronegative females in Rayong Province, Thailand
Conference Name: Fifth Internatinal Congress on AIDS in Asia and the Pacific
Conference Location: Kuala Lumpur, Malaysia
Abstract: OBJECTIVE: To study the frequency banding profile of indeterminate HIV Western blot in seronegative females. This is important background information for distinquishing infection from antibodies induced by vaccines.
METHODS: Sera from one hundred-twenty females attending family planning clinic and screened by HIV ELISA as negative were tested with western blot (Bio-Rad) test. The interpretation criteria for anti-HIV-1 Western blot test recommended by the U.S. Centers for Disease Control were used in the study.
RESULTS: Among 120 seronegative sera , 112 (93%) were found to be negative by Western blot and 8 (7%) were indeterminate The patterns of the indeterminate Western blot were all based on antibody to gag protein (p24=6 sera and p17=2 sera).
CONCLUSIONS: More than 90% of seronegative sera were found to be negative by Western blot. Indeterminate banding patterns occurred infrequently and were specific to the gag proteins.

Reference Type: Conference Proceedings
Record Number: 95
Author: Guarnacci, T; Birx, D; Rungruengthanakit, K; Brown, A; Dax, E; Sittisombut, N; deSouza, M
Year: 1999
Title: Quality Assessment Program Implementation in Support of Clinical Assays for Phase I/II HIV Vaccine Trials Thailand
Conference Name: Fifth International Congress on AIDS in Asia and the Pacific
Conference Location: Kuala Lumpur, Malaysia
Abstract: Introduction: A quality assurance program (QAP) is essential for evaluating the performance of laboratories involved in a multi-center HIV vaccine trial. Our laboratory developed a QAP to support the exclusion/inclusion criteria as mandated by the study investigators' vaccine protocol.

Objectives: To monitor the performance of collaborating laboratories involved in the clinical assessment of HIV vaccine trial participants; to evaluate the preparedness of participating laboratory sites; to identify problems and provide support in the form of investigative and corrective action; and to evaluate consistency of quantitative result reports from sites using differing technologies.
Methods: Proficiency testing panels (PTP) were distributed to 6 sites on a quarterly basis. These panels consisted of samples for qualitative (syphilis/urinalysis dipstick/urine pregnancy) and for quantitative evaluation (serum ALT/creatinine and CBC - at 2 sites). PTP results were analyzed by the AFRIMS site and result reports were provided to all participants. Quantitative results were subjected to statistical analysis based upon intra/inter-laboratory performance as referenced by single parameter testing.
Results: Since the initiation of the proficiency testing program (PTP) in May 1997, eight (8) PTP have been distributed. Subsequent result evaluations revealed >90% concordance of qualitative and quantitative results between reporting sites. Reasons for result variations were dependent upon several factors including over analysis of samples by participating laboratories and use of improper device accessories.
Conclusion: Proficiency testing panels ensure that accurate and consistent result reports are generated from participating laboratories and that supporting documents, in the form of standard operating procedures, are followed. This PTP program also provides an excellent forum for the discussion and resolution of current and potential issues relating to all laboratories involved.

Reference Type: Conference Proceedings
Record Number: 100
Author: Thapinta, D; Jenkins, R; Chaddic, C; Chinaworapong, S; Sonsatapornkul, P; Naksrisook, S; Ruangyuttikarn, C
Year: 1999
Title: Volunteer Recruitment for Prophylactic HIV- I Vaccine Trial in Thailand
Conference Name: Fifth International Congress on AIDS in Asia and the Pacific
Conference Location: Kuala Lumpur, Malaysia
Abstract: Background: Assess demographic and behavioral characteristics of volunteers for prophylactic HIV vaccine trial in Thailand. Evaluate factors that may relate to retention through the recruitment process. The trial was a randomized, double-blind, placebo controlled evaluation of Chiron HIV Thai E gp 1 20/MF59, administered with HIV SF2 gp 120 in healthy HIV seronegative adults.
Methods: Data were collected during screening process prior to enrollment into the trial. Those who passed all biomedical and behavioral screening procedure completed a test of understanding before being offered enrollment in the trial.
Results: 56.5% of volunteers presented at screening, was enrolled into the trial (divided among 4 sites), of these screened 56.3% was male, 43.7% was female, median age was 33 (range: 14-60) occupations are very varied. Past HIV testing was reported by 3 9 % . Past history of blood donation was reported by 3 7.5 3 %. The most common motivations for interest in the trial was to help society and a desire to do something good. Demographic variables varied by site. Ile test of understanding was successfully passed by 90% of those screened. After enrolling, only 1.04% of

volunteers are dropped out from the trial. None of them have HIV infected and all have low risk behavior.
Conclusion: Recruitment was succeeded 56.5 % male more than female. Our rate was very low, no HIV infected and they have low risk behavior. That means the personnel have to have very good counseling and education with volunteers.

Reference Type: Conference Proceedings
Record Number: 87
Author: Leelamanit, W; Chuethong, J; Boonyom, R; Panuim, S; Polonis, VR; Nitayaphan, S
Year: 1999
Title: Genetic variability of subtype E HIV-1 protease from therapy-naive Thai patients
Conference Name: Joint International Tropical Medicine Meeting
Conference Location: Bangkok, Thailand
Abstract: HIV-1 isoloates are genetically classified into different subtypes according to env and pag coding squencences. In Thailand, approximately 90% and less than 10% infected subjects are infectied with HIV-1 clades E and B, respectively. Since any mutation in the HIV- protease domain many generate protease-resistant strains, information on in vivo sequence divesity of the protease of the clade E will lead to a better understanding of the susceptibility of the enzymes to inhibitors of HIV isolates in this region. We have analyzed 30 protease-coding sequences form 3 therapy-native individuals. Briefly, polymerase chain reaction technique was used to amplify protease coding regions form the proviral DNA isolated from peripheral blood monouclear cells. The data indicated that the protease sequences of subtype E were unique and clearly different from HIV reference strain for clade B. Protease variants analyzed from the same subject were nearly homogenerous and different form patient to patient. Additionally, some random mutations could be observed in certain isolates. These critical variations may change the enzymatic activity of the protease, thus creating drug-resistant strain. We plan to extend our sequence analyses and determine the enzymatic activity of these protease variants.

Reference Type: Conference Proceedings
Record Number: 86
Author: Brown, A
Year: 1999
Title: Future HIV Vaccine Trials Supported by the U.S. Army in Thailand
Conference Name: International Conference on HIV vaccine development; Global, Regional and Thailand update,1999
Conference Location: Bangkok, Thailand
Abstract: Collaboration between scientists of the Royal Thai and U.S Armies led to the isolation and sequencing of Thai E viruses in the early 1990s. From these strains, which showed relatively narrow genetic diversity, were derived the rgp 120 vaccines manufactured by Chiron and VaxGen for testing in Southeast Asia. A similar isolate, from the WHO Network, is now the basis of both the ALVAC and rgp 160 constructs made for testing here by Pasteur-Merieus-

Connaught. As a result of these collaborative efforts, more candidate HIV vaccines are available in Thailand for subtype -specific testing than in any other onon-B epidemic region of the world. The U.S . Army collaboration with the Thai AIDS Vaccine Evaluation Group (TAVEG) is assessing both subunit protein and live vector constructs. The 4-site phase I/II trial of the Thai E rgp 120 cancidate from Chiron Vaccines (reviewed by S. Nitayaphan) is nearing completion. A protocol is under review to under review to carries the gene for a Thai E gp120, as well as genes for the transmembrane portion of gp41 (IIIB). It is proposed that different Env protien "boosts" (more than one manufacturer's candidate) will be assessed with this single "prime". ALVAC-HIV will be given to healthy HIV-negative adults at 0,1 ,3 and 6 months; the "boost" will be given at 3 and 6 months. Safety and immunogenicity (antibody and CTL0 will be deteminde and assessed against predetermined milestones prior to advance to a phase III trial.
The challenge of carrying out such an efficacy trial among. Thai heterosexuals has grown with the success of public health efforts in lowering HIV incidence. The U.S. Army-TAVEG collaboration is approaching a Go/No Go decision regarding the feasibility of such a trila. Multiple cohort studies are ongoing to provide current data to inform this decision. Thailand has led the developing world in phase I and II trials of HIV vaccine candidates and the phase III trial in Bangkok IVDUs is likely to be the first completed efficacy tril of an HIV vaccine. A phase III vaccine tril in adults at risk of heterosexual transmission of HIV poses a great challenge to Thai public health leadership and the U.S . Army-TAVEG collaboration.

Reference Type: Conference Proceedings
Record Number: 96
Author: Polonis, V; de Souza, M; Chanbancherd, P; Chantakulkij, S; Nitayaphan, S; Birx, D; Brown, A
Year: 1999
Title: Characterization of Neutralization Sensitivity of HIV-1 Subtype E and Thai B' Viruses Using V3 Serotyped Plasma Pools
Conference Name: Fifth International Congress on AIDS in Aisa and the Pacific
Conference Location: Kuala Lumpur, Malaysia
Abstract:
Background: Assessment of primary isolate neutralization continues to be a goal in laboratories involved in the development and testing of HIV-1 vaccines; clinical trials testing products from both subtypes B and E are currently ongoing in Thailand. Given the genetic diversity of HIV and the existence of multiple clades, a panel of characterized HIV-1 isolates with known neutralization sensitivity will provide a valuable tool for assessing the immunogenicity of candidate vaccines.
Methods: An HIV-1 neutralization (NT) assay was performed using primary peripheral blood mononuclear cells, clinical isolates from clades B' and E, and plasma pools. Plasmas collected from Thai subjects were serotyped by V3 loop peptide ELISA and plasmas were pooled. One E pool contained plasmas collected prior to 1995 and the other contained E plasmas collected in 1998. The NT assay employed a p24 reduction format; 80% reduction of p24 production was used as cut-off for NT sensitivity.
Results: Forty-five Thai viruses of know syncytium inducing (SI) phenotype were characterized as sensitive (NT by 2 or more plasma pools), moderate (NT by one pool), or completely resistant

to neutralization. Contrary to what has been observed with N. American subtype B viruses, a greater proportion of the sensitive viruses (83%) were of the NSI phenotype and 53% of the resistant viruses were SI. The subtype E viruses were characterized as 23% NT sensitive, 36% moderate and 41% resistant to NT.
Conclusions: The development of a panel of well-characterized subtype B and E plasmas and isolates for exchange amongst collaborating laboratories in Thailand and S. east Asia will facilitate our standardization of neutralizing antibody assays used for vaccine immunogenicity studies. An understanding of the evolution of the biological properties these subtypes, such as syncytium inducing phenotype and neutralization sensitivity, may provide insight into the design of international vaccines for HIV.

Reference Type: Conference Proceedings
Record Number: 83
Author: Chanbancherd, P; Jugsudee, A; Thanomklom, S; Limpairojn, N; Julanato, P; Thienamporn, P; Markowitz, LE; Polonis, VR; de Souza, MS; Brown, AE
Year: 1999
Title: Assessment of WHO strategy for HIV testing using results of the Royal Thai Army (RTA) serosurveillance data
Conference Name: APMMC IX
Conference Location: Bangkok, Thailand
Abstract: For HIV serosuvelliance in low prevalence (<10%) populations, the WHO suggests the serial use of 2 ELISA/rapid /simple serologic assays. We assessed the positive predictive value (PPV) of this alternative HIV testing strategy in a cohort of Thai males. 111,639 serum samples from males (age range 21-22 yr) entering service with the Royal Thai Army in 1996-1997 were screened with tow different HIV-1/2 3 rd generation ELISAs (1st , Abbott Laboratories; 2nd, Viroonostika Uniform II Organon Teknika), the second was performed only if the first was reactive. Sera reactive in both assays were tested further by HIV-1 Western blot (WB) (Diagnostic Biotechnology, Singapore) and results were interpreted according to the CDC/ASTPHLD criteria. A total of 2,839 (2.5%) sera were reactive in the first ELISA: 2,322 (81.8%) of those were also reactive in the second ELISA. The number of true positives, negatives, and indeterminates, as determined by WB, were 2,195, 62 and 65 respectively. The prevalence of HIV infection in the studied group was 2.0%. The false positive rate of the two sequential 3rd -generation ELISAs was 0.58% (644/111,639). The PPV of Abbott testing alone was 77.3% (2,195 of 2,839), wheras the PPV of two sequential ELISAs was 94.5% (2,195 of 2,322). This study confirmed that sequential use of two 3rd -generation ELISAs increased the PPV of HIV testing . Six per thousand HIV false positive resutls might occur in a low prevalence population that used two sequential 3rd -generation ELISA tests alone. Such a false positivity rate (<1%) makes sequential use of 2 ELISAs a very good tool for surveillance in saving cost, but not for clinical diagnosis due to a finite number of misdiagnosed individuals.

Reference Type: Conference Proceedings
Record Number: 101
Author: Rangsin, R; Sirisopana, N; Chiu, J; Nelson, K; Khamboonruang, C; Beyrer, C; Markowitz, L
Year: 1999
Title: HIV incidence among young Thai men 4-6 yeras after discharge from The Royal Thai Army
Conference Name: Fifth International Congress on AIDS in Asia and the Pacific
Conference Location: Kuala Lumpur, Malaysia
Abstract: Background: Studies of HIV- I incidence among young Thai men living in Northern Thailand could give a reliable estimate of the recent epidemic in Thailand. This study is designed to describe the HIV-1 incidence, 4-6 years after discharge from the Royal Thai Army (RTA) who were conscripted into the army in 1991-1992 and discharge in 1993-1994.
Methods: A retrospective cohort study was conducted of 195 conscripts who were HIV- I seronegative at the time of discharge from the RTA in 1993-1994. The study population was randortily chosen from the populations of military conscription from the 6 upper Northern provinces who contrived living in this area after their military discharge. A follow-up survey was done of these former soldiers, with behavioral interviews and phlebotomy, between Nov. 1998 and June 1999. HIV-1 scrostatus was determined by ELISA and confirmed by Western Blot. T. pallidurn infection was determined by Rapid Plasma Reagin(RPR) and confirmed by TPHA. CD4 count was determined by flow cytometry and viral load was quantitated using the Roche Amplicor (version 1.5) assay. Incidence was determined using person-time analysis.
Result: As of May 31, 1999. 181(92.8%) of 195 subjects have been identified. 7(3.9%) was deceased and 147 were enrolled into the survey. The median age at time of discharge was 23 years. The median age at time of the follow-up was 28 years. The median time of follow-up was 5 years. All the subjects provide a total of 740 person-years of follow-up; 4 of 147 were HIV positive. The HIV- I incidence rate was 0.54 per 100 person-years. All of the HIV-positive men were asymptomatic; median CD4 count was 352/ul. HIV- I RNA was detected in each of 4 HIV-1 seropositives (rage 10742-84282 copies/mL). Also 4(2.8%) men were positive for active syphilis infection.

Reference Type: Conference Proceedings
Record Number: 85
Author: Brown, A; Nitayaphan, S
Year: 1999
Title: An HIV Vaccine Development Strategy for Southeast Asia
Conference Name: Cambodia National HIV/AIDS Conference
Conference Location: Phnom Penh, Cambodia
Abstract: The pandemic of HIV/AIDS consists of multiple foci with distinct epidemiological characteristics. Among the approximately on million Southeast (SE) Asians infected with HIV, subtype E is the predominate subtype of the virus. This subtype, a recombinant virus comprised of a genotype A core(gag) gene and a distinct. envelope (env) gene, became broadly epidemic in Thailand beginning in 1989, and more recently in Cambodia and other parts SE Asia.

The diversity of SE Asian subtype E viruses is relatively narrow, probably reflecting its recent introduction. Since specific immune responses to HIV infection may be subtype specific, the test of a subtype E-derived candidate vaccine in SE Asia would provide an optimal test of vaccine concept. To develop and test HIV vaccines in Thailand, a consortium of industry (Chiron Vaccines, Pasteur-Mérieux-Connaught), academic (Mahidol and Chiang Mai Universities and military (United States and Royal Thai Army Medical Departments) medicine has been formed called the Thai Vaccine Evaluation Group (TAVEG). In 1995-96, the TAVEG carried out a phase I/II trial of a subtype B rgp 120 candidate vaccine in Thai adults, and confirmd it to be dafe and immunogenic. Buidling upon that first trial, Chiron Vaccines has modified the vaccine to match th eEnv protein of subytype E viruses. Currently, the TAVEG is carrying out a phase I/II trial of this subtype E vaccine in 380 healthy audlt volunteers in Bangkok and Chiang Mai. While this subunit protein vaccine is designed to elicit antibody responses, planning is moving ahead for a new trial of a "prome-boost" combination vaccine. A live-vectored vaccine will "prime" the immune response and the rgp 120 protein will "boost" this response. For this purpose, canarpox vectors (ALVAC) containing multiple HIV genes (gag/pol/env) orignially disigned as genotype B constructs, have been modified by Pasteur-Mérieux -Connaught to Contain a subtype E env gene.

HIV vaccines efficacy cannot currently be predicted by studies in animal models nor by laboratory assasy. Thus, to develop a vaccien for protection againts HIV, it is crucial to carry out clinical trials in humans. Sequential trials from phase I and II to phase III are carried out under protocols which are fully reviewed both scientifically and ethically. This process is time consuming, since insuring the safety of volunteers is the first priority. But it si through this careful process fo cliniciall trials that the hope of the region and the world for an HIV vaccine willl eventually be realized.

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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	Suchin	Poolgird	Supervisor Assistant
7	Samran	Kongsua	Animal Care Taker Technician
8	Thonglor	Detkokao	Animal Care Taker Technician
9	Sawang	Sripakdee	Support Supervisor Section
10	Phatcharaphon	Jaikla	Operator Equipment
11	Samruay	Jecksaeng	Operator Equipment
12	Dechmongkol	Onchompoo	Operator Equipment
13	Manop	Pooyindee	Operator Equipment
14	Choosri	Sangsri	Guard
15	Komgrit	Ekkachart	Admin. Assistant
16	Jarin	Keawjarat	Section Supervisor
17	Sarawut	Komjalern	Section Supervisor
18	Anchalee	Tungtang	Section Supervisor
19	Surayuth	Srigaewin	Support Technician
20	Somkid	Tosawong	Support Technician
21	Manas	Gaewsurin	Support Technician
22	Ekkapop	Srichumnan	Animal Facility Engineer
23	Wittawat	Sangalee	Lab Animal Technician
24	Mana	Saithasao	Veterinary Technician
25	Yongyoot	Gongkaew	Animal Technician

Glasswares Worker

No.	*Name*	*Surname*	*Position*
26	Sawadi	Boonnak	Glasswares Worker Supervisor
27	Charan	Kajeechitr	Glasswares Worker
28	Thongchai	Duangkaew	Glasswares Worker
29	Boonthum	Jamjang	Glasswares Worker
30	Komson	Boonnak	Glasswares Worker

Administration

No.	*Name*	*Surname*	*Position*
31	Sutthida	Srijan	Admin. Clerk
32	Weerasak	Yeephu	Computer Technician
33	Sompol	Boonnak	Computer Technician
34	Daungjai	Lumson	Data Entry

35	Barnyen	Permpanich	Nurse
36	Nipat	Promchart	Air-Conditining Repairman
37	Russama	Jittawisuthikul	Admin. Clerk
38	Thongsuk	Munmuenpom	Chauffer
39	Somchai	Putsang	Chauffer
40	Pattrapan	Jullasing	Information Technologist
41	Puwanai	Sangsri	Audio-Visual Assistant
42	Mongkol	Puramas	Chauffer
43	Surapol	Ogpai	Chauffer
44	Yaowalux	Kitkungwal	Secretary

HIV PERSONNAL

1	Kritika	Singharaj	Admin. Assistant
2	Narongrid	Pongpakdee	Data Entry
3	Nucharee	Thongsaen	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	Yaemutai	Data Entry
9	Sithinan	Bunyatub	Data Entry
10	Suchart	Thepsanan	Data Entry
11	Supin	Pankote	Data Entry
12	Wisut	Lokpichart	Programmer
13	Oranuch	Supapyarn	Special Project Tech
14	Prapatcha	Chitsunthornrat	Special Project Tech
15	Kornchanok	Panjapornsuk	Medical Technologist
16	Kampol	Puapuek	Research Assistant
17	Vinai	Kaneechit	Research Assistant
18	Sirivajra	Ekapirat	Medical Technologist
19	Sutchana	Tabprasit	Medical Technologist
20	Suchart	Chuangpho	Cleaner/Messenger
21	Apichat	Sudathid	Technician
22	Athaya	Sukchamnong	Technician