Special Article

The Development of HIV Research Laboratories in the Royal Thai Army Medical Department

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The development of HIV research laboratories at the Armed Forces Research Institute of Medical Sciences (AFRIMS), Royal Thai Army Medical Department in supporting of HIV-1 vaccine trials in Thailand was implemented in 1991. The collaboration between AFRIMS, Royal Thai Army Medical Department, and the US Military HIV Research Program with the ultimate goal to conduct the HIV-1 vaccine trial phase III. The HIV serology lab was set up for surveillance program in military recruits. Then, there was a need to strengthen more on the existing laboratories by training personnel to cope with the confidentiality of the lab results, specimen processing and data management which are critical. Later on, the necessary laboratory for measuring of vaccine immunogenicity was developed, such as lymphoproliferation assay. Additionally, a molecular biology lab was also developed. The HIV research laboratory management must include an ability to deal with some problems, such as late specimen receiving, fluctuating of power supply, technical staffs maintained. Good laboratory practices and safety must be strictly implemented. Communication network among facilities also played an important role in HIV laboratory strengthening at AFRIMS.

Keywords: *HIV research laboratory development, HIV-1 vaccine trials, Armed Forces Research Institute of Medical Sciences*

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The HIV-1 pandemic has grown to become one of the greatest infectious diseases threat to human health and social stability that the world has ever encountered. In Thailand, AIDS is also one of the critical public health and military personnel problems. The first reported cases of AIDS in Thailand occurred in 1984 in a homosexual male[1]. However, extensive transmission of HIV-1 began in 1988 among IDU, in Bangkok in 1989[2]. Thus, a biannual sentinel surveillance was conducted among blood donors, IDU, female commercial sex workers and pregnant women by the Thai Ministry of Public Health[3]. In addition, the Royal Thai Army conducted HIV-1 screening of young men conscripted to serve with the Royal Thai Army (RTA)[4]. In the year 2004, from a total population of 61 million, it was estimated that 1,074,155 persons were infected with HIV since the beginning of the epidemic. It was also estimated that 19,500 new infections would occur during 2004 compared to 143,000 new infections in 1990[5]. However, the development of preventive vaccine is the best hope of controlling the HIV epidemic.

The HIV laboratories for evaluation of HIV vaccine candidates in phase I/II and III trials in Thailand was implemented in 1991 by collaboration between AFRIMS, RTA and the US Military HIV Research Program. The authors established a research

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laboratory infrastructure and technology to evaluate HIV vaccine candidates, determine immune correlates of protection against HIV infection and provide high quality data on the basic research and molecular epidemiology. This basic knowledge may be helpful in reducing HIV transmission to others.

Royal Thai Army Medical Department and Collaboration

The Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand originated in 1958 when a group of scientists from Thailand and the United States established the Southeast Asia Treaty Organization (SEATO) Cholera Research Laboratory. It was renamed the SEATO Medical Research Laboratory when the mission was expanded in 1961 and the Laboratory became the AFRIMS upon dissolution of SEATO in 1977[6]. For more than 40 years AFRIMS, RTA has worked closely with the United States providing outstanding research, training and technology transfer. In 1991, collaboration between AFRIMS, RTA and the US Military HIV Research Program was established to conduct HIV-1 vaccine trials in Thailand. The first project of Thai—US collaboration was the study of the prevalence and incidence of military recruits who were annually screened for HIV. The HIV serology laboratory was the first laboratory set-up at AFRIMS in order to detect HIV antibody in HIV-infected military recruits. However, this project was also in collaboration between AFRIMS and the Army Institute of Pathology (AIP), Bangkok. After that, the other HIV laboratories were developed and established at AFRIMS. In 1991-1992, the US component Department of Retrovirology was established at AFRIMS[7] and then, the Joint Clinical Research Center (JCRC) was established at Phramongkutklao Hospital (PMKH), Bangkok in 1992[8]. The joint refers to the collaboration between AFRIMS Thai and US Components, PMKH, Walter Reed Army Institute of Research (WRAIR) and Henry M Jackson Foundation for the Advancement of Military medicine (HMJF). In 1992, a study of the natural history of HIV infection in both prevalent and incident cases was initiated. The Research Institute of Health Sciences (RIHES) at Chiang Mai University initiated collaboration with the HIV research Program, AFRIMS, to form the ARVEG (AFRIMS-RIHES Vaccine Evaluation Group) in 1994. Later on, two sites of Mahidol University, Siriraj Hospital and the Vaccine Trial Center within the Faculty of Tropical Medicine joined the collaboration in 1997. The four-site collaboration was named the Thai AIDS Vaccine Evaluation Group (TAVEG). Because infrastructure is essential for laboratory development, HIV laboratory development raises a question of what infrastructure-capacity building is needed?. The laboratory infrastructure AFRIMS built a training laboratory for personnel in laboratory techniques and good laboratory practices (GLP), developed laboratory biosafety manuals, guidelines and standard operating procedures (SOPs), laboratory quality assurance (QA) program, monitoring and audits, specimen processing, storage and shipment, secure data record, storage and management, collaboration and laboratory network, and organization and participation in the workshops.

Training Laboratory Personnel

There was a need to strengthen some existing laboratories to conduct HIV-1 vaccine trials in Thailand, training personnel for high technology transfer from international collaboration was started. Our scientists and laboratory personnel visited the HIV laboratory at WRAIR Rockville and then laboratory technology was transferred from the USA to AFRIMS. In addition, the laboratory personnel of AFRIMS were trained in biosafety, GLP, research methodology, relevant immunological, molecular and virological tests, storage, specific assays and data management. GLP includes general: source of laboratory infections, laboratory hazard, laboratory workers rights and duties in relation to safety measures. The basic approach to the management of risk protective associated with blood borne pathogens is to practice universal precautions, which presuppose that all blood, body fluids, and other specimens collected from patients are infectious and handled by using appropriate personnel protective equipment.

The assays established in HIV research laboratory of AFRIMS, RTA and Department of Retrovirology for support of HIV vaccine trials especially measuring vaccine immunogenicity and basic research are HIV serology assays: enzyme-linked immunosorbent assay (ELISA) and western blot; immunophenotyping of white blood cells by Flow Cytometry: CD4/CD8; intracellular cytokine cell staining (ICC); cytotoxic T-lymphocytes (CTL) killing assay; lymphocyte proliferation assay (LPA); ELISPOT Assay; binding antibody assay by ELISA; neutralization assay, functional assay; HIV culture; HIV coreceptor usage assay; and HIV molecular assays: viral load, PCR, cloning and sequencing. However, the epidemiology studies and natural history cohort studies were also supported by our laboratories.

Natural History Cohort Studies

According to RTA conducting HIV-1 screening of young men conscripted to serve with the RTA, the studies of prevalent and incident cases were initiated in 1991. AFRIMS, RTA collaborated with AIP and the WRAIR set up ELISA and Western Blot assay and established a diagnosis algorithm to support these studies[9]. The first study carried out was the temporal trend of HIV seroprevalence among young men entering the Royal Thai Army: 1989-1990[10]. Later, the incidence of HIV-1 infection among young men in Thailand was reported [11]. The prevalence data generated from screening of young men conscripted to serve with the RTA and the sentinel surveillance of MOPH have produced the world s most complete characterization of a national HIV epidemic. The authors see that the laboratorys play a key role in identifying HIV-infected persons so it can help decrease the rate of new infections. In addition, prevalent and incident cases enrolled by JCRC were studied for natural history[8]. Basically, most specimens were tested CD4+ T cell count, HIV genotyping by PCR and viral load. Many publications and abstracts of significant findings have resulted from these studies[12-19].

In 1992-1993, lymphoproliferation assay and Immunophenotyping assay by Flow cytometry were established at AFRIMS, RTA. Lymphoproliferation assay measuring in vitro functional cell-mediated immune responses demonstrated that asymptomatic HIV-1 subtype CRF01_AE infected Thais[12]. Also, similar to the delayed-type hypersensitivity skin test assessing in vivo cellular immune responses showed that frequency of anergy was inversely related to CD4 cell count; 38% of patients with CD4 counts of 0 to 200 cells/ml were completed anergic[13]. For immunophenotyping assay by flow cytometry, besides, measurements of CD4, CD8 T cell counts, and other subpopulations of white blood cells, the authors detected intracellular cytokines in peripheral blood of HIV-1 infected Thai children[14].

Furthermore, HIV molecular lab was also established in 1992-1993 at AFRIMS, RTA. Based on the difference in V3 loop between subtype B and CRF01_AE, the authors found correlation of V3 loop serotypes and genotype done by PCR[15]. With transfer of HIV full-length genome sequence assay from WRAIR to AFRIMS, RTA, the authors found CRF01_AE/B recombinants in Thai patients[16].

In addition, the authors studied immune correlation of HIV-1 disease progression and HIV-1 evolution in subtype CRF01_AE —infected Thais and found that rising neutralizing antibody titers and anti-CD4/gp120BS were associated with slower disease progression[17]. Anti-p24 titers significantly decreased over time in more rapid progression and cross-clade ADCC activity against HIV-1 subtype B of sera from HIV-1 subtype CRF01_AE infected Thais was observed[17]. Also, there was no association between serum β-chemokines and disease progression[18]. In V3 region, GPGQ motifs were found in all slower progressors and predicted phenotype of slower progressors were all NSI, while V3 region of progressors evolved more rapidly. There was deglycosylation of *env* V3 sequence of HIV-1 subtype CRF01_AE infected progressors, whereas it was conserved in slower progressors[19].

HIV Vaccine Trials

The ultimate goal of HIV laboratory development at AFRIMS is support of Phase III HIV vaccine trials. However, Phase I/II and III HIV vaccine trials[8] were conducted by AFRIMS and multi-sites. For Phase III HIV vaccine trial, it has been conducted: as follows

1) Phase I trial of Biocine HIV SF2 gp120/MF59, subtype B vaccine in seronegative Thai volunteers (RV99), by AFRIMS, RIHES and enrollment in August 1995.

2) Phase I/II double blind, placebo-controlled study of the Chiron Biocine HIV Thai E gp120/MF59 vaccine administered alone or combined with the Chiron Biocine HIV SF2 gp120 antigen in healthy HIV-seronegative Thai adults (RV114), by AFRIMS, RIHES, VTC, Siriraj and enrollment in November 1997.

3) ALVAC-HIV (vCP1521) priming with either oligomeric gp160 TH023/LAI-DID or Chiron HIV Thai E (CM235) gp120 [+/- SF2 gp120] boost (RV132) by RIHES, Siriraj and enrollment in January 2000.

4) ALVAC-HIV (vCP1521) priming with Vaxgen gp120 B/E (AIDSVAX B/E) boost (RV135), by AFRIMS, VTC and enrollment in March 2000.

5) Boost injections with higher dose gp120/MF59 subtype E antigen in RV114 volunteers previously immunized (RV114A)), by AFRIMS, VTC and enrollment in November 2000.

6) Phase III trial using Aventis Pasteur s live recombinant ALVAC-HIV (vCP 1521) priming with VaxGen gp120 B/E (AIDSVAX B/E) boosting in HIV seronegative Thai adults, by AFRIMS, Thai MOPH, Mahidol University and US Military HIV Research Program and started in October 2003.

The safety and immunogenicity data of HIV vaccine trials on various products were generated by

HIV laboratory of AFRIMS RTA collaborated with HIV laboratory of Department of Retrovirology, US component and several publications and abstracts were published[20-29]. The Biocine HIV SF2 gp120/MF59, subtype B vaccine of phase I vaccine trial in seronegative Thai volunteers was found to be safe and immunogenic[20]. Anti-gp120 antibody[21] and lymphoproliferative responses[22] were induced and persisted for at least 4 months. Cross-clade lymphoproliferative responses to clade E was also induced[23]. Safety and immunogenicity of combinations of recombinant subtype E and B HIV-1 envelope gp120 vaccine in healthy Thai adults was demonstrated[24,25]. Subtype E-specific neutralizing antibodies were detected in 85% of vaccines.

For the prime-boost vaccine combinations which induces humoral and cellular immunity, they were found to be well tolerated and safe[26-28].

Phase III trial using Aventis Pasteur s live recombinant ALVAC-HIV (vCP 1521) priming with VaxGen gp120 B/E (AIDSVAX B/E) boosting in HIV seronegative Thai adults are based among young adults in Chonburi and Rayong provinces[29]. The HIV laboratory measurement at AFRIMS accredited by CAP performed the specimen from 16,000 volunteers and phase III trial is an on going study.

Developed Laboratory Biosafety Manuals, Guidelines and Standard Operating Procedures (SOPs)

SOPs are needed to ensure consistency of laboratory operations in the pursuit of quality, train new laboratory personnel and troubleshoot problem areas. SOP must include: title, principle, specimen requirements, reagents or media, supplies, equipment, calibration, quality control, step-by-step instructions, calculations, reporting results, procedure notes, limitations of methods, a troubleshooting or back-up plan, references, effective date and signature of laboratory director. Importantly, the monthly quality improvement (QI) meeting is set-up at AFRIMS to correct and solve our laboratory problems. All complaints and resulting corrective action must be documented.

Laboratory Quality Assurance (QA) Program, Monitoring and Audits

The AFRIMS, RTA and Department of Retrovirology has implemented a rigorous Quality Assurance (QA) program to ensure compliance with Good Clinical and Laboratory Practices. The QA Program adheres to standards described in the National Committee for clinical Laboratory Standards Guidelines (NCCLS) and participates in the proficiency panel testing program of the College of American Pathologists (CAP). The authors first CAP accreditation was achieved in September 2002 for HIV serological and Flow cytometry laboratory that is the first clinical laboratory in Thailand to receive this accreditation. However, the authors maintained CAP accreditation in 2004 and will continue for CAP accreditation in the next 2 years.

Specimen Processing, Storage and Shipment

1. Specimen processing

Since specimen processing is an important aspect of the pre-analytical testing, the lab personnel are well-trained to perform basic specimen processing (centrifuging, aliquoting, and storing) to complex specimen processing including capability for performing lymphocyte isolation and cryopreservation. In addition, SOP s for specimen processing are written to ensure that all specimens are collected and processed in accordance with the approved instructions specific for each protocol.

2. Storage of materials including vaccine, reagents, specimen and waste under controlled circumstances

The room storage equipment is very important so it should be in a secure reliable, temperature controlled and fully monitored facility. Our laboratories have access to 4^{o}C refrigerators, -40^{o}C, -80^{o}C, and liquid nitrogen tanks to store the biological materials. Importantly, we manually measure the temperature and record each freezer daily.

3. Shipment of storage materials to and from sites:

AFRIMS laboratory has the capacibility to ship materials to and from sites as follows: specimens, radioisotopes, equipments, and materials

However, the shipment of dangerous goods is the responsibility of the shippers so\the authors should identify, prepare, classify, pack, label and document all shipments that contain biological reagents and dangerous goods. Importantly, the authors have to follow the regulation of the International Air Transport Association (IATA) if ship them by air route. Moreover, the authors have to ensure shipments are received on time.

Secure Data Record, Storage and Management

Secure data recorded confidentially and only authorized personnel shall enter the archives and keep the documents at least 2 years following the date on

which the study is completed. Bioethical regarding counseling, informed consent and confidentiality are the issues that are definitely needed.

Collaboration and Laboratory Network[30]

In 1994, HIV laboratory networks of Thailand was established by the Department of Communicated Disease Control, Ministry of Public Health, AFRIMS, Siriraj Hospital Mahidol University, Chiang Mai University, Chulalongkorn University, Khon Kaen University and Songkhla University[5]. The authors need to promote laboratory networks and conduct meeting and training workshops to transfer technology that is appropriate. The one mission of the laboratory networks of Thailand is strengthening the existing HIV laboratory in Thailand toward standards that include consistency, reproducibility, trace and efficiency.

Organization and Participation in the Workshops

AFRIMS contributed to organize and participate in HIV workshops in order to transfer laboratory technology, learn more technology, and also exchange ideas, for examples: WHO Workshop on Advance Techniques in screening of HIV-1 Genetic Variability in Thailand at the Department of Microbiology, Faculty of Medicine, Siriraj Hospital, Thailand[31]. February 1995; South East Asian and Western Pacific regions Quality Assurance Workshop by National Serology Reference Laboratory Australia in Bangkok, Thailand. January 1997; Workshop on Nucleic Acid Amplification for Diagnosis and Monitoring of HIV-1 Infection at Department of Microbiology, Faculty of Medicine, Siriraj Hospital, Thailand[30]. February 1997; International HIV/AIDS Summer School, University of Saskatchewan, Saskatoon, Canada. August 1997; Annual meeting of AIDS/Flow cytometry in Thailand, started in 1993 to the present.

Laboratory Problems Encoutered and Solution

1. The laboratory personnel

Prophylactic immunizations such as HBV vaccine immunization should be required for all laboratory personnel when benefits of vaccination outweigh the rights such as hepatitis B. The safety program provides medical surveillance to laboratory personnel for infections that may result from experimental agents encountered in the performance of routine duties. However, the vaccine administered to the lab personnel or the refusal of vaccination by the employee should be documented.

2. Technical staffs maintained

Sometimes, the change or quiting the jobs of technical staff is one problem the authors have encountered. However, understanding of our staff, work benefit and progression should be promoted. Good laboratory practices must be strictly concerned.

3. The specimen problems

The conditions of the specimens which must be avoided are lipaemic, hemolyzed and contaminated sera; repeated freeze-thawed sera and heat-inactivated — may cause falsely reactive ELISA.

Guidelines for collecting samples and for evaluating submitted specimens are, therefore, essential because acceptance of improper specimens for analysis may lead to errorneous results.

Late specimen receiving and the number of specimens that are a small number of samples to large numbers (phase I/II to phase III HIV vaccine trial) are also specimen problems.

3. Equipment problems

Incubator-poor air circulation, temperature and fluctuating of power supply are the problems to be solved.

4. Operator/technical problems

Improper timing of incubations can give low or high absorbance readings.

5. Communication network among research facilities

The policy must address the communication of results between laboratories and the physician which regard to results. Additionally, other research facilities such as transportation, clinical research center and laboratories have to communicate all the time.

Thailand was one of the first countries to define HIV/AIDS as a national security threat. The National AIDS committee of Thailand with the initiation and the cooperation of the World Health Organization and the Joint United Nation Program on HIV/AIDS has set the development of HIV Vaccine as a priority and implemented the National plan for HIV/AIDS Vaccine Development. Since 1993 the plan has catalyzed and facilitated the implementation of numerous HIV/AIDS vaccine related activities, including the organization of multiple consensus-building, technical cooperation, building the capacity of Thai scientists, institutes and national research networks of HIV laboratories[5]. According to the support of the National plan for HIV/AIDS Vaccine Development, HIV

laboratory development, one of the critical parts of HIV vaccine development, could be its successful mechanism.

The critical factors facilitating success of HIV laboratory development are the responsibility and dedicated laboratory personnel, excellent collaboration laboratory network, both national and international collaboration, the well-established laboratory infrastructure and good communication network among research facilities. Thirteen years experience in HIV research laboratory of our personnel at AFRIMS, could serve as the basis and model of laboratory development in Thailand. Thus, they are very essential and useful, especially infrastructure building, strengthening of the technology capability and technology transfer, not only among laboratories in Thailand but also in other developing country's laboratories. The successful HIV research laboratory development results from many factors but the main of which is willingness to control HIV epidemic in Thailand especially HIV vaccine development.

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การพัฒนาห้องปฏิบัติการเอชไอวีในกรมแพทย์ทหารบก

ทิพย์วรรณ ชื่นจิตร, สุจิตรา สุขวิทย์, จริยาณาฏ เกวี, ขวัญใจ วิพุทธิกุล, จิราภา เอี่ยมศิลา, สุชชนา แทบประสิทธิ์, Mark de Souza, ณรงค์ฤทธิ์ ศิริโสภณา, สรชัย นิตยพันธ์, Arthur E Brown, เชิดชัย ชื่นจิตร

การพัฒนาห้องปฏิบัติการเอชไอวีของสถาบันวิจัยวิทยาศาสตร์การแพทย์ทหาร กรมแพทย์ทหารบก เพื่อสนับสนุนการทดสอบวัคซีนเอดส์ในประเทศไทยนั้นได้เริ่มขึ้นเมื่อปี พ.ศ. 2534 โดยความร่วมมือของสถาบันวิจัยวิทยาศาสตร์การแพทย์ทหาร และโปรแกรมงานวิจัยเอชไอวี กองทัพสหรัฐอเมริกา วัตถุประสงค์หลักของการศึกษาวิจัยร่วมกันนี้คือ การศึกษาทดสอบวัคซีนเอดส์ระยะที่ 3 สำหรับห้องปฏิบัติการแรกที่ได้จัดตั้งขึ้น คือ ห้องปฏิบัติการเอชไอวีด้านน้ำเหลืองวิทยา เพื่อรองรับงานการเฝ้าระวังการติดเชื้อเอชไอวีในทหารกองประจำการ สิ่งสำคัญประการแรกของการพัฒนาห้องปฏิบัติการ คือ การพัฒนาฝึกฝนบุคลากรทางด้านห้องปฏิบัติการ รวมทั้งขั้นตอนต่างๆ ที่สำคัญ เช่น การเตรียมตัวอย่างตรวจ และระบบการจัดการกับข้อมูลต่างๆ จากนั้นจึงได้พัฒนาห้องปฏิบัติการเพื่อตรวจวัดการกระตุ้นภูมิคุ้มกันของวัคซีน เช่น การตรวจวัดการตอบสนองโดยการแบ่งตัว และเพิ่มจำนวนของลิมโฟซัยท์ นอกจากนี้ห้องปฏิบัติการอณูชีววิทยาได้พัฒนาเช่นกัน การจัดการห้องปฏิบัติการเอชไอวีจะต้องรวมถึงความสามารถในการแก้ปัญหาที่เกิดขึ้นต่างๆ เช่น การได้รับสิ่งส่งตรวจที่ส่งมาล่าช้า ระบบไฟฟ้าที่ไม่สม่ำเสมอหรือไฟดับ การทำให้นักวิจัยคงทำงานที่สถาบันไม่เปลี่ยนงาน ระบบคุณภาพห้องปฏิบัติการและความปลอดภัยจะต้องปฏิบัติอย่างเคร่งครัด เครือข่ายของการติดต่อสื่อสารในแต่ละส่วนมีบทบาทสำคัญในการทำให้ห้องปฏิบัติการเอชไอวีของสถาบันวิจัยวิทยาศาสตร์การแพทย์ทหารเข้มแข็งด้วยเช่นกัน