CHAPTER I

INTRODUCTION

1. Background and Rationale

Acquired immunodeficiency syndrome (AIDS) is one of the current world's biggest health issues. It is caused by the human immunodeficiency virus (HIV). There are 9 HIV-1 subtypes and 14 different circulating recombinant forms (CRF) have been identified.[1] The HIV epidemic has spread worldwide. Thailand is one of the epidemic areas in Asia. It was estimated that by the year 2000, 1 million Thais living with HIV infection.[2] There are 2 major strains of HIV-1 found in Thailand.[3] According to the study, 96 per cent of the HIV-1 isolated from Thai men in 1992 and 1995 were carried circulating recombinant form A/E (CRF01_AE), the rest carried subtype B'.[4] In contrast, in developed countries where most of the scientific and medical study results generated, subtype B is the most common strain reported. In addition, several factors such as HIV subtypes, host factors, or other co-factors may affect the natural history of HIV-1. Thus, data from Western cohorts may not be all applicable to HIV infection in Thailand, particularly in the HIV/AIDS vaccine research and development.

Vaccine strategies aimed at blocking virus entry have so far failed to induce protection against the heterologous viruses because of the HIV-1 envelope strain variability.[5-7] Thus, the control of viral infection and the prevention of disease progression may represent a more achievable goal of HIV vaccine strategies. There is a hypothesis about the observation that the magnitude of HIV replication during the initial phase of HIV infection determines HIV disease progression and outcome.[8]
In addition, clinical and experimental observations imply that control of viral expansion during the initial acute viral infection is a key point for intervention to negate chronic viremia and clinical progression.[9-10] Transactivator of transcription or Tat protein is, an essential regulatory protein of HIV-1, is one of the tempting targets for such control. The Tat protein was chosen because Tat is produced early after

infection and is essential for virus replication and infectivity.[11-12] Prior specific vaccination against Tat protein is designed to remove the evidently essential basis for the massive multiplication of virus, which fosters uncontrollable production of viral variants surviving and eventually destroying immune defenses.[13-14] Although a Tat vaccine cannot block virus entry, the immune response to Tat may control virus replication and transmission.[13-15] As a result, the infection could be confined and progression to AIDS could be blocked.

The immune responses against HIV infection are attributed by two major types of lymphocytes: B lymphocytes, which secrete soluble antibodies into the bloodstream, function against free-floating HIV proteins cell free virions and thereby mediate humoral immunity; and T lymphocytes, which are involved in the immune responses requiring direct cell-cell contact, resulting in the cellular immunity against viral infected cells. There are two subtypes of T cell, which are responsible for these functions, the killer or cytotoxic T cell (CTL cell) and the helper T cell (Th cell). Several studies demonstrated that CTLs are important protective immunity against HIV infection.[16-21] It is able to kill the target cells displaying epitopes of foreign antigen on their surface. It is also able to secreate and/ or to stimulate cells to secrete a variety of cytokines, which influence the function of other cells, involved in body defense and promote a variety of nonspecific body defenses.[20,22] CTLs have been shown to produce HIV viral inhibitory factors.[24-25] It is established that HIV-specific CTLs appear early in the course of infection and are temporally associated with the clearance of culturable virus from blood. In addition, such CTLs are commonly found in high numbers during the asymptomatic phase of infection and decline with progression to AIDS.[26-29] Reports of such cases include health care workers exposed to contaminated blood, infants born of HIV-1-infected mothers, and also subjects repeated exposed to HIV-1 from unprotected intercourse, including homosexuals, prostitutes and sexual partners.[30-34] These studies suggest that CTLs may have the capacity to prevent transmission of HIV. Furthermore, reports on virus escape from HIV-1-specific CTLs responses clearly indicate that CTLs exert pressure on virus replication *in vivo*.[35-36]

The detection of Rev and Tat-specific CTL precursors (CTLp) inversely correlate with rapid disease progression is in agreement with the hypothesis that CTL against proteins that are important for early viral transcription and translation are importance in protection from rapid disease progression.[10] Tat is not only essential for high-level HIV replication by increasing transcription initiation and elongation but also has a non-transcriptional role as well: Tat released by HIV infected cells readily enters and activates uninfected cells, rendering them susceptible to HIV infection.[37-40] There are several biological reasons for believing that an immune response directed against Tat may be particularly effective in controlling HIV. First, this protein is less able to change than other parts of HIV; some parts of Tat might not be able to change without making a virus, which could not sustain an infection.[41] Also, Tat is produced early in the life cycle of HIV before the virus has had time to suppress immune responses through other mechanisms.[42] Although Tat-specific CTL responses are elicited in HIV-infected patients and in non-human primate models.[13-15, 43-45] The number of stidies of specific CTL epitopes within Tat so far has been limited.

In this study, HIV-1 CRF01_AE Tat protein was targeted for the screening of CTL responses by gamma interferon (IFN-γ) -Enzyme-linked immunospot assay (Elispot) with truncated and overlapping synthetic peptides of Tat. Elispot is currently a method of choice in the preliminary screening of dominant CTL responses in population studies. There are several advantages of the assay, for instance highly sensitive, easy to perform, and low-technique setting. In addition, the IFN-γ-Elispot assay has shown to be a reliable method to map optimal CTL epitopes.[46-47] It makes testing of a wide spectrum of truncated synthetic peptides and screening of a large number of HIV-infected individuals can be performed easily and effectively.[48-54] Furthermore, Elispot has been shown to correlate well with other methods, such as intracellular staining, tetramer staining, and the classical chromium release assay.

The characteristics of the predominant virus that causes the HIV-1 epidemic in a certain geographic area and also the genetic background of the population, through the distribution of common HLA class I alleles, might impact dominant CTL responses in the general population. There is a limited number of CTL studies an HIV-1 non-B subtypes and of CTL epitopes mapping among Asian population

including Thais. Our study, therefore has identified and characterized HIV-1 CRF01_AE Tat-specific CTL responses that might provide some specific and additional useful data for the research and development of CRF01_AE Tat-based vaccine.

Research Questions

Primary Question

: Do HIV-1 infected Thais generate HIV-1-specific CTL responses against the Tat protein, and how is it?

Secondary Question

: Which parts of the Tat protein do their CTLs response to?

Research Objectives

: Characterization of HIV-1-specific CTL responses against the HIV-1 Tat protein.

: To find the parts of the Tat protein that CTL recognizes and shows CTL response to.

Limitation of the Study

This study is an *in vitro* study based on HIV-1-infected PBMC, and the results from this study may not correspond completely to the *in vivo* setting.

Key Words

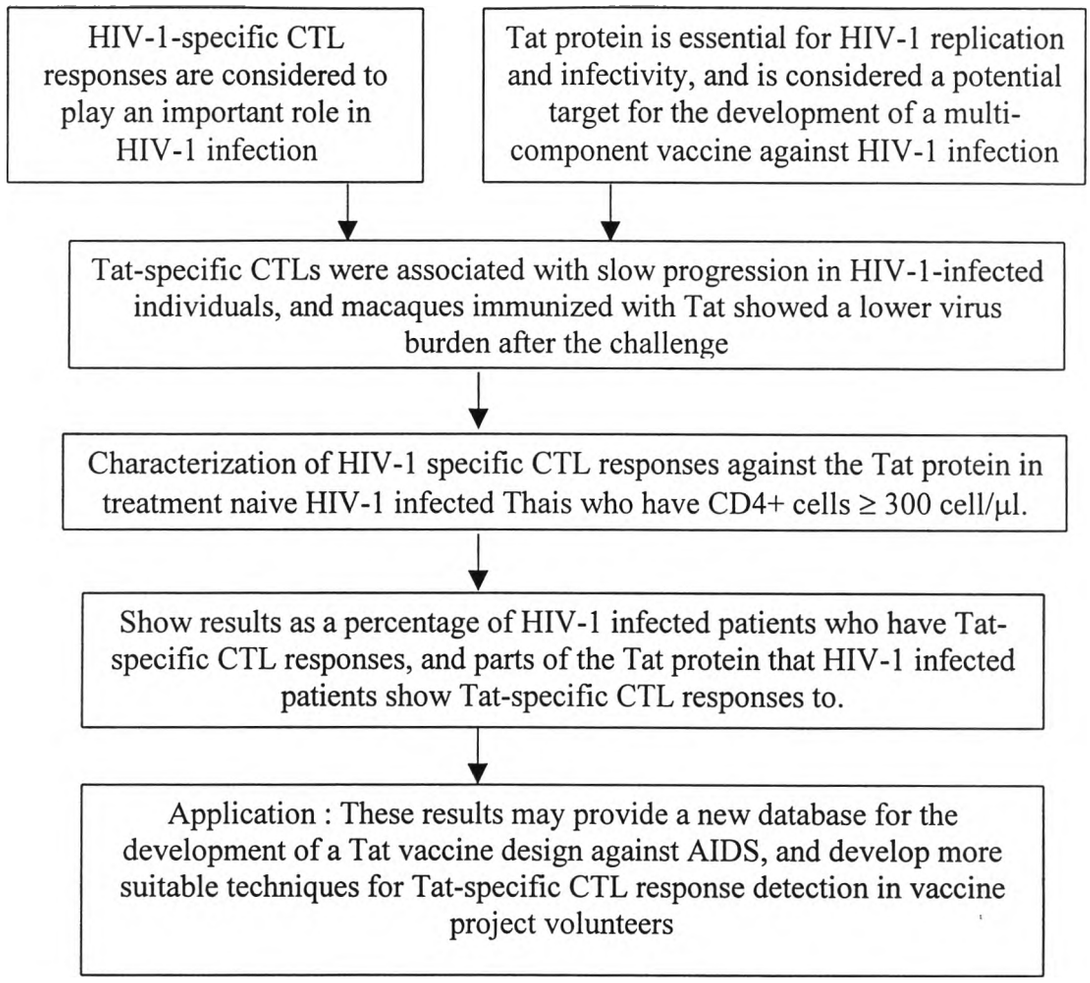
CTLs

Epitope

HIV-1

Tat

Conceptual Framework



Expected Benefits & Application

1. From our findings, we hope to learn whether HIV-1 infected Thais have HIV-1-specific CTL responses against the Tat protein, and the extent of these responses they have.
2. Be able to identify immunodominant CTL epitopes in the Tat protein among Thai Patients
3. The results may provide useful new database for future analyses of the HIV-1 Tat protein.
4. The data may be helpful for the development of preventive and therapeutic Tat-based vaccines against AIDS that are suitable for Thai population.

Research Methodology

Study Subjects

Twenty antiretroviral treatment naive HIV-1 infected Thais, age ≥ 20 with CD4+ cells count ≥ 300 cells/ μl were included. All provided written consent. The study was approved by the Chulalongkorn Medical Institutional Review Broad (IRB), Faculty of Medicine, Chulalongkorn University.

Study Methods

1. Truncated Tat Peptides Design and Synthesis

In this study, we designed and synthesized 10 truncated HIV-1 CRF01_AE Tat peptides of 17 to 21 amino acids that overlapped by 10 amino acids. Tat exon I sequence was based on the isolates virus from 12 Thai patients attended our clinic and the sequence of HIV-1 isolate CM 240 from Thailand 1990, complete genome (accession number U54771). The complign PPC MacMolly® Tetra, Version 3.5-March, '97 program was used to find the consensus sequence of 42 HIV-1 CRF01_AE isolate from Thailand 1990-1995. The consensus sequence was used for Tat exon II peptide design. The truncated Tat peptides were synthesized at Natural and Medical Sciences Institute at the University of Tuebingen (Germany)

2. Separation of Peripheral Blood Mononuclear Cells (PBMC)

PBMCs were separated from 40 ml of heparinize whole peripheral blood by Ficoll-Hypaque density gradient centrifugation.

3. Enzyme-linked Immunospot Assay (Elispot) for the Detection of Human Gamma-interferon.

3.1 For the Tat Elispot screening: Each subject was screened by pooled Tat peptides (2 pools of peptides, each pool contains 5 peptides).

3.2 Identification of Tat specific epitope by Elispot: The pooled peptide that showed IFN-γ-Elispot positive of screening was then further tested with each individual peptide.

4. Enumeration of IFN-γ SFU and Data Collection

The IFN-γ spots were counted by a stereomicroscope and are expressed as an average spot-forming unit (SFU) per million PBMCs of the duplicate wells.

5. Statistic Analysis

The number of specific IFN-γ-secreting T cells was calculated by subtracting the negative control value from the established SFU count. Results of 100 or more SFU/10^6 input cells were considered positive, and the negative controls were always <100 SFU/10^6 input cells. Tat-specific CTL responses were shown as a proportion of the patients who have responses to at least one of the Tat peptides.

Administration and Time Schedule

Phase	Process	Time schedule (0 2 4 6 8 10 12 14 16 18)
1	Preparation phase	→
2	Peptide design and ordering	→
3	Subjects collection and analysis	→
4	Data collection and analysis	→
5	Conclusion and thesis writing	→