CHAPTER I

INTRODUCTION

Human papillomaviruses (HPV) are members of the *Papovaviridae* Family and the *Papillomavirus* Genus. The HPV genome consists of circular double-stranded DNA approximately 8 kilobase pairs (kb). The virus is nonenveloped virus and has icosahedral symmetry. HPV particles are 50 – 60 nm in diameter. The HPV genome encodes six early proteins and two late proteins on a single strand of DNA. Early proteins have been shown to be necessary for the replication and the transformation. The E1 and E2 are viral regulatory proteins that are involved in viral DNA replication and in controlling viral transcription. The E4 protein is a late protein expressing in the terminally differentiated keratinocytes. The oncogenic proteins E5, E6 and E7 proteins have transforming activities (1, 2), whereas the function of E3 and E8 are not known. The two late proteins, L1 and L2 encode major capsid protein and minor capsid protein, respectively (3).

HPV have a high degree of tissue specificity. HPV infect only surface squamous epithelia of the skin or mucosa (2). After infection, HPV will replicate as episome in nucleus of epithelium cell. The replication of HPV resulting in virion progeny is controlled by cell growth and differentiation. Besides episomal stage, the viral DNA is able to integrate into the host cell chromosome. The integration may lead to break in the E1 – E2 region resulting in the loss of transcriptional control of oncogenic E6 and E7 genes (3, 4). E6 binds to tumour suppressor protein, p53, promoting its rapid degradation via the ubiquitin pathway (5-7). E7 can interact with retinoblastoma tumour suppressor protein, pRb, causing the release of E2F and cell cycle progression (5-8). E5, on the other hand, associates with the epidermal growth factor receptor (EGFR) resulting in increased ligand-dependent activation of EGFR and enhanced EGFR-mediated mitogen-activated protein (MAP) kinase activity (9).

The classification of HPV types is based on degree of relatedness of genomes. A discrete HPV type is defined as having less than 90% nucleotide sequence homology within the L1 region when compared to other known types (2). To date, over 100 different HPV have been described (4, 10, 11). HPV are also grouped according to

potential for malignant transformation. Two groups have been classified based on risks of causing cervical intraepithelial neoplasia (CIN) and cervical cancer (CaCx) for example, high-risk group (HPV-16, 18, 31, 33, 35, 39, 45, 51, 56, 58 and 66) and low-risk group (HPV-6, 11, 42, 43 and 44) (12-15). Recent evidence in CaCx specimens has shown that HPV was presented in 99.7% of cases (16). Of the 15 high-risk HPV types isolated from cervical carcinomas, HPV-16 is the most frequently detected, occurring in over 50% of CaCx (12, 17, 18). Furthermore, the WHO's International Agency for Research on Cancer (IARC) classified HPV infection as "carcinogenic" to human (HPV-16 and 18) (4). The prevalence of HPV infection varies depending on geographical location. In the previous study, the detection rate of HPV-16 (36.6%) in Asian and African patients with CaCx (19-22) was lower than that reported in Europe and U.S.A. (60 - 84%) (23-25). Whereas, in Thailand, HPV-16 was the most common type in CIN patients representing 44.04%, followed by HPV-18 (15%), HPV-33 (9.33%) and HPV-11 (4%) (17, 26).

Cervical cancer is the second leading cause of cancer deaths in women worldwide. More than 450,000 cases are diagnosed each year (27). The National Cancer Institute of Thailand reported that the incidence of CaCx was the most common among cancers in women (http://www.nci.go.th/statisti.htm). Aetiology and development of CaCx were associated with the persistent of high-risk HPV infection. The frequency of HPV infection in CaCx patients was found to be higher than that reported in healthy control women (28). Moreover, studies in women persistently infected with high-risk HPV types, HPV-16 and 18, were showed that these women were more likely to develop CaCx (28, 29). This finding highlighted the significance of HPV genetic variation in the pathogenesis of CaCx. Indeed, in a study analysing HPV-16 E6 and E7 sequence in women with normal histology or CIN I, CIN II/III and invasive CaCx confirmed that HPV intratypic variations were associated with an increased risk for developing CaCx (30). In naturally occurring HPV-16 E7 variation, several studies were found to be specific mutation at pRb binding site (amino acid 21 – 29). The variations of E7 may be effect to virus virulence or divert of biological and biochemical properties (31-35). Recently, the diversified HPV-16 variants have been grouped into major phylogenetic clusters: European (E), African-1 (Af1), African-2 (Af2), Asian (As), Asian American (AA) and North American-1 (NA1) (36).

HPV-specific cytotoxic T lymphocytes (CTL) were believed to play an important role in the pathogenesis of HPV-associated disease. The protective role of HPV-16 E6 and E7-specific CTL was demonstrated in HPV-16 infected patients who did not developed CIN in a number of studies (37-39). In one study, the percentage of patients demonstrating HPV-16 E6- and E7-specific CTL was higher in a group of women with HPV-16 infection who did not develop CIN than in a group of women with HPV-16 infection who developed CIN. This study suggested that CTL response played a role in disease protection (37). Most HPV-CTL studies, however, were carried out in Caucasians who might have been infected with HPV within non-Asian cluster. In addition, these studies demonstrated the HPV-CTL responses in the context of Caucasian Human Leucocyte Antigen (HLA). In order to understand HPV-specific CTL responses in Thai donors, we demonstrated the HPV-E7-specific CD8+ T cell responses in HPV-infected Thai women with precancerous and cancerous cervix by ELISpot assay. Information obtained from this study would be essential for an effective HPV vaccine development.