CHAPTER 3

CONCLUSIONS AND PERSPECTIVES

HBV infection remains the most common infection and a major health problem worldwide. It is estimated that 350 to 400 million people are chronically infected with HBV (2-7); of whom three quarters reside in Asia and the western Pacific region (8, 9). In these areas, the majority of HBsAg carriers have been infected at birth or in early childhood (13).

Thailand, a country with high prevalence of chronic HBV infection, has been one of the countries hardest hit by the HIV-1 pandemic. It is estimated that over half a million people are currently living with HIV; of whom 9% are co-infected with HBV. We have addressed in this HIV-HBV co-infected population 3 questions of public health interest: i) what is the residual risk of perinatal transmission of HBV among HIV-HBV pregnant women in the context of EPI, ii) what is the prevalence and impact of occult HBV infection among these women and iii) what is the long-term efficacy of 3TC-containing HAART on HBV infection. The common point to these 3 questions relates to the possible occurrence of mutations of the *pol* or *S* genes of HBV and their potential negative impact on diagnosis, response to anti-HBs immunoglobulins and/or vaccine and antiviral therapy.

The most effective means to decrease HBV burden and HBV disease complications is to prevent mother-to-child transmission of HBV by combining passive and active immunoprophylaxis. However, 5 to 10% of newborns acquire HBV infection despite early injection of HBIg and adequate HBV vaccination. In the first part of this work, I have assessed the prevalence of HBV mother-to-child transmission (MTCT) and characterized the distribution of virus variants that were transmitted from HBV/HIV-1 co-infected women to their children. I have found a 5% transmission of HBV for which different mechanisms could account for. In one-third of transmitted mothers, maternal wild-type virus was transmitted from mothers with high level of HBV DNA. In the remaining cases, maternal HBV variants accounting either for 20% or more of the maternal viral population or minor variants were transmitted. I have identified several HBsAg mutations: sK122R, sI126IT, sI126M+P127S, and sT131N+M133T+T140I+S204R for which the following questions arise: Can these variants escape anti-HBs response and eventually induce vaccine failure? A study has been initiated in collaboration with Camille Sureau, head of the molecular virology laboratory of the French Institute for Blood Transfusion, Paris, France, to ascertain the capacity of those HBV variants to escape *in vitro* neutralization by anti-HBs antibody. The research programme involved preparation of hepatitis D viral (HDV) particles expressing the different variants of HB envelope proteins found in five mother and infant pairs (Table 3.1). According to the methodology described by Wu and co-workers (507, 508), HDV particles were produced by co-transfection of Huh-7 cells with plasmids containing cDNA of HDV and plasmids containing HBV full length genome. To date, we have produced nine HDV particles harboring the mutations of interest characterized in 3 mother-infant

pairs T0022, T1395 and T1550. The presence of HBsAg and HDV RNA was checked in culture supernatants for each particle (Figure 3.1). Then, infectivity of these particles was tested on HepaRG cells by detection of intracellular HDV RNA and compared to that of HDV wild type particles (Figure 3.2). The next step will be to analyze the susceptibility of such HDV particles to be neutralized by anti-HBs antibody from HBV naturally-infected individuals or from HB vaccinated subjects. We have selected 30 sera from HB vaccinated people and 30 from HBV naturally-infected individuals with high anti-HBs titers and the neutralization assays are on process. Understanding the causes of HB vaccine failures will help to develop new HBV vaccines appropriate for HBV endemic countries such as Thailand and other South-East Asian countries or develop other interventions to decrease perinatal transmission of HBV and accelerate the eradication of HBV infection.

Table 3.1 Amino acid differences on *pre-S1*, *pre-S2* and *S* genes of selected clones issued from each mother and child pair.

		Pre-S1				Pre-S2	S-gene											
Amino acid position		10	27	35	38	49	2	5	8	10	31	40	47	115	122	126	146	188	200
T0022	Maternal clone #1	Q								G					K				Y
	Infant clone #1	K								E					R				C
	Infant clone #2	K								G					R				Y
T1395	Maternal clone #1					T									K		N	P	
	Infant clone #1					A									R		D	L	
	Infant clone #2					T									R		N	P	
T1550	Maternal clone #1		G	G			G							T		I			
	Infant clone #1		D	R			E							T		T			
	Infant clone #2		D	R			E							N		T			
N0625	Maternal clone #1		G													I			
	Infant clone #1		D													T			
N9149	Maternal clone #1				P			A	L		R	N	V						
	Infant clone #1				S			S	P		S	S	G						

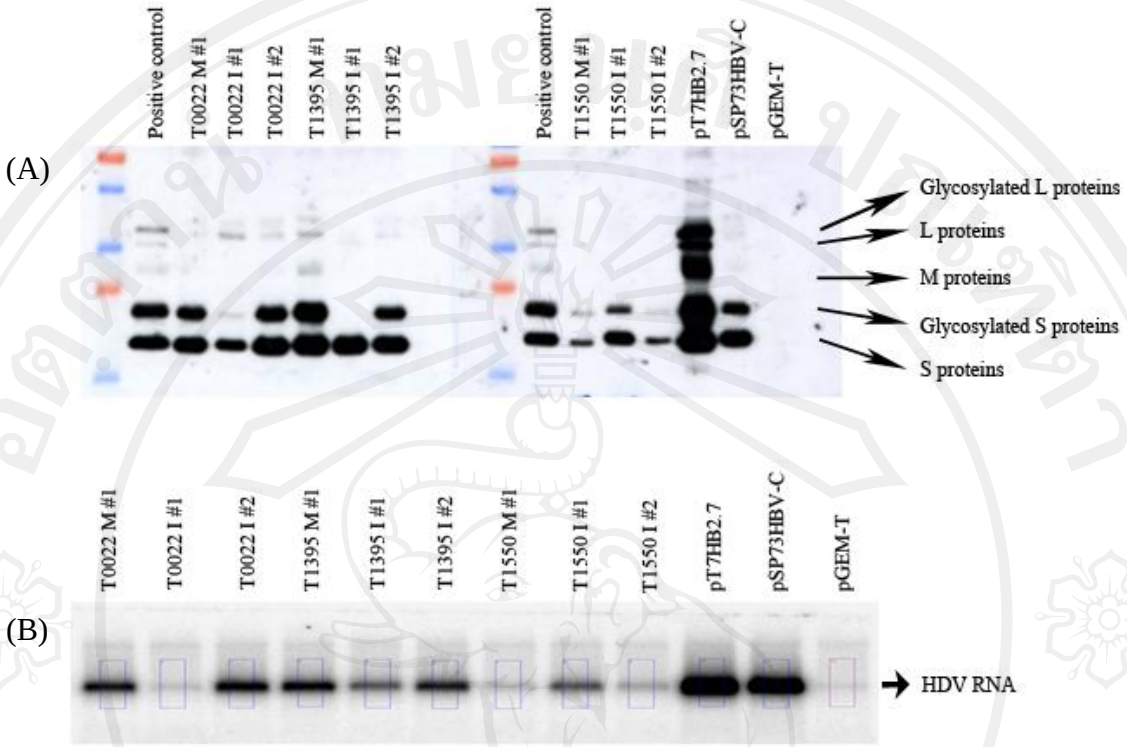

Figure 3.1 Production of HDV particles harboring HBsAg mutants after co-transfection with plasmids containing HBV and HDV genomes. HuH-7 cells were co-transfected with 2000 ng of HBV-plasmids and 1000 ng of HDV plasmids. Culture supernatants were collected every 2-3 days and pooled before HBsAg/HDV RNA detections.

(A) HBsAg detection in culture supernatants (4-8 days post-infection) by western blotting.

(B) Detection of HDV RNA in culture supernatants (10-27 days post-infection) by northern blotting.

Positive control, *plasmid containing an HBV genotype D full length genome****; pT7HB2.7,*** *plasmid containing the pre-S1-pre-S2-S gene that can direct the expression of the S, M, and L proteins - it includes the HBV promoter upstream of the pre-S1 region for expression of the mRNA for the L protein, the HBV promoter within the pre-S1 region for expression of the M and S mRNAs, and the HBV polyadenylation signals;* ***pSP73HBV-C,*** *plasmid containing HBV genotype C full length genome (serotype adrq+);* ***pGEM-T,*** *empty vector of expression;* ***M #1,*** *maternal clone #1;* ***I #1,*** *infant clone #1.*

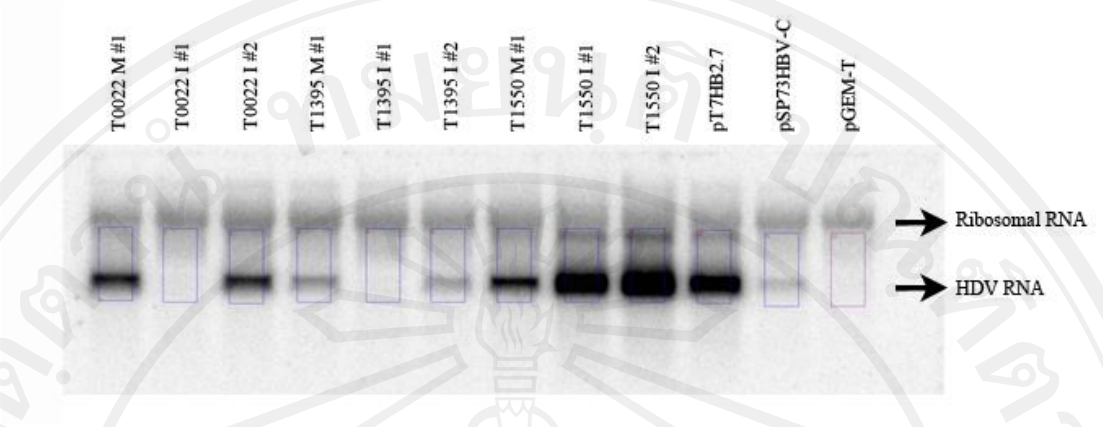

Figure 3.2 Infectivity testing of produced HDV particles harboring HBsAg mutants. HepaRG cells were infected with same amount of produced HDV (182 units). HDV RNA was detected at days 7 post-infection by northern blotting.

Another obstacle to HBV eradication is the high number of chronically HBV infected subjects, who are not yet treated because of the limited access to anti-HBV treatment or are not aware of their HBV infection. These chronically HBV infected subjects may thus represent a major source of viral spread. However, not all hepatitis B virus infections are symptomatic and even routine serological markers can miss the diagnosis of HBV disease. Thus occult HBV infections have been recently described and are defined by positive HBV-DNA in the absence of serum HBsAg (376). Occult HBV infection has been a major concern for blood banks or organ transplantation. One consequence of occult HBV infection in HIV-infected patients is the possible re-activation of HBV infection, particularly after immune suppression (230). Occult HBV infection has been frequently found in individuals with isolated anti-HBc serologic profile (377, 392). In the second part of this study, we have first reported that the prevalence of isolated anti-HBc in HIV pregnant women throughout Thailand was 14%. About 5% of these women became positive for HBsAg with a different

HBsAg-test kit though very low levels. The prevalence of occult HBV infection among women with confirmed HBsAg-negative and isolated anti-HBc was 24%; 94% of women with HBV occult infection had HBV DNA level <100 IU/mL and 6 % had HBV DNA between 101 to 1000 IU/mL. The prevalence of HIV-infected pregnant women presenting isolated anti-HBc/occult HBV infection was low (2.6%). I have also showed that older age, birth in northern region, low CD4 count and exposure to HCV were independent factors associated with isolated anti-HBc serologic profile. Sequencing of S gene was successful for only 2 samples and multiple mutations (sS117I, sT118K, and sR160K) were identified in one sample. None of the women with isolated anti-HBc and occult HBV infection transmitted HBV to their infants.

In Thailand, the first line recommended antiretroviral regimen for treating HIV-infected patients in Thailand was until recently a fixed dose combination including 3TC. It is usually admitted that treatment of HBV in HIV-HBV co-infected patients leads to the emergence of HBV mutations associated with resistance to 3TC at a frequency of 15-20% per year. As the consequence of overlapping genes of HBV, this resistance may lead to the occurrence of HBsAg mutations. In the last part of this study, I have evaluated the HBV virological response in HIV/HBV co-infected patients receiving 3TC-containing HAART and analyzed in patients with HBV breakthrough the selection of virus with 3TC resistance mutations and their possible consequence on mutation of S gene. I have demonstrated that all HBeAg-negative patients and 63% of HBeAg-positive HIV-HBV co-infected patients achieved long-term HBV DNA suppression while on 3TC-containing-HAART. The cumulative rates of HBV DNA suppression were 91%, 84%, and 68% at 1, 3, and 5 years,

respectively. The rate of HBeAg and HBsAg loss at the last visit were 87% and, 19% respectively. Surprisingly the rate of resistance mutations to 3TC was lower than expected from the data published in the literature. Indeed mutations were found in 3 patients: 1 had the rtM204I mutation, 2 had the rtV173L+L180M+M204I triple mutation pattern and none of the 3TC induced-mutations identified resulted in mutation of the virus *S* gene. My results showed that 3TC exert an activity on HBV longer than what was reported in studies conducted in Europe and that negative HBeAg may be a used to predict the good response to 3TC. This study provides information useful for the management of co-infected patients in resource-limited countries where the vast majority of co-infected patients are currently receiving 3TC.

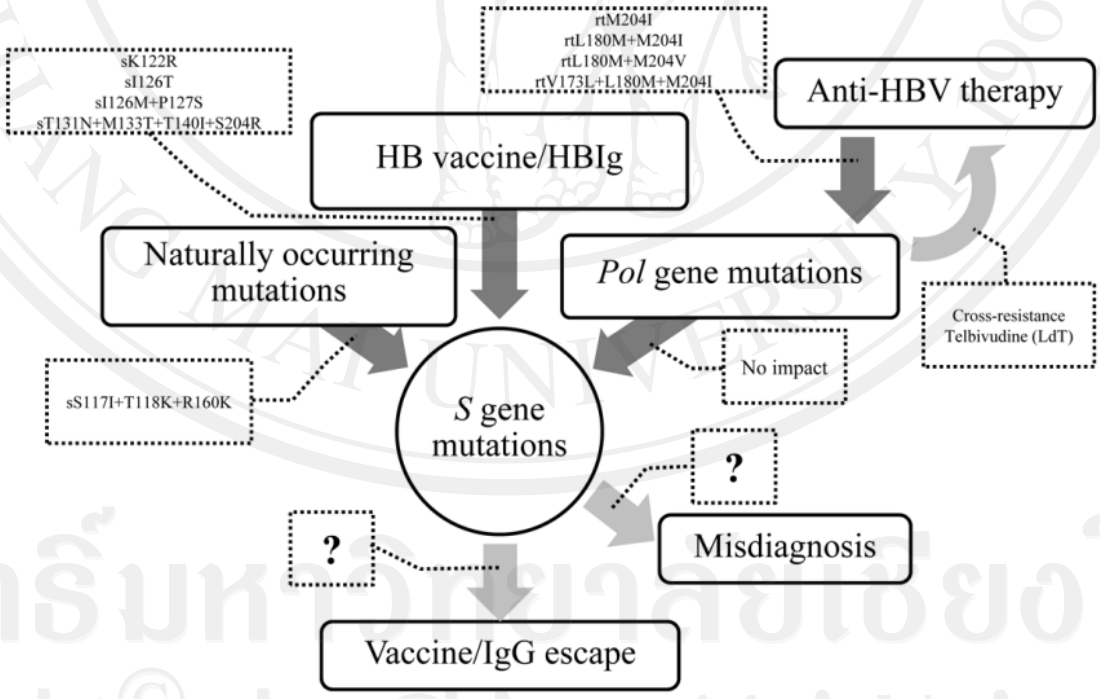

Figure 3.3 Overall figure drawn from the study results

Overall conclusion obtained from this study is described in Figure 3.3. Hepatitis B viral mutants can emerge naturally in HIV-HBV co-infected patients or as a result of selection pressure from either immune response or treatment options. However, in this study, I could not demonstrate the impact, on *S* gene, of *Pol* gene mutation induced by 3TC-containing HAART. Cross-resistance to another anti-HBV nucleoside analogue, telbivudine, was identified. Mutations that occur within the immunodominant epitopes of HBsAg allow mutant virus to survive and propagate in the presence of a neutralizing immune response, while wild-type virus is reduced to undetectable levels. The most important point is that virus harbouring these HBsAg mutations may transmit and infect others individuals who have already been vaccinated or it may present as false-negative results in some immunoassays. However, these issues need to be further investigated.

All the knowledge gained form this work may be useful for researchers and public health specialists to develop new strategies or interventions to decrease HBV burden as well as improve treatment of HIV-HBV co-infected patients in Thailand and also in other Southeast Asian countries. Combined effort will be needed to reach the goal of eradication of HBV.