CHAPTER 5

Cellular Immune Responses after Different Hepatitis B Vaccination Regimen in HIV-infected Individuals

5.1 Introduction

HBV is one of the most serious infectious virus that causes hepatitis B liver diseases. As HBV and HIV share similar routes of transmission, co-infection with HBV is more common in HIV-infected individuals than in the general population [280-282]. The progression of HBV-related liver disease in HIV+ individuals is more accelerating than patients with chronic HBV infection alone [283, 284]. Therefore, HBV prevention by vaccination in HIV+ individuals is strongly recommended [285-287]. However, the efficacy of HBV vaccine to induce protective antibody in HIV+ individuals is critically low compared to HIV– general population [229, 288-292]. Several studies have reported the improvement of responses to HBV vaccine by using higher dose [227, 228], increasing frequency [211, 228], and increasing dose and frequency [177, 228, 229]. Responsiveness to HBV vaccination is defined by the generation of anti-hepatitis B surface antigen (HBsAg) more than 10 IU/mL (levels presumptive for seroprotection) after vaccination [293].

B cells, the precursors of antibody-producing plasma cells play an important role in the humoral immune response. Antigen-specific B cells development requires help from CD4+ T cells and dendritic cells in order to from germinal centre in B cell follicle of secondary lymphoid tissues, where somatic hypermutation of immunoglobulin variable region genes and selection of high-affinity B cells occur [294]. B cells that acquire adequate T cells help then differentiate into long-lived memory B cells or plasma cells [294]. CD4+ T cells also play a critical role in the establishment of CD8 T cell cytotoxic activity which important against viral infection. Besides neutralizing antibodies which play an important role in protection against HBV infection, T cell

immune responses is considered necessary for sufficient control and clearance of HBV [134]. In contrast to widely studied serological response, CMI response to HBV vaccination is less well understood, especially in HIV+ population. In this study, we investigated cytokine production and expression of degranulation marker, CD107a, of T cells in response to *in vitro* recombinant HBsAg stimulation after different strategies of HBV vaccination in HIV+ individuals compared to standard vaccination in healthy controls.

5.2 Methods

5.2.1. Vaccination and blood collection

The Hepavax-Gene vaccine (Hepavax-Gene®, Berna Biotech Korea Corp, Incheon, South Korea) containing non-infectious inactivated recombinant HBsAg was used in this investigation. HIV+ individuals were randomized (1:1:1) by block of six into 3 groups. They were given different doses and frequencies of vaccine as shown in table 5.1. Group 1 was defined as the "Standard dose group"; subjects were vaccinated with 20 μg of HBV vaccine at days 0, 28 and months 6. Group 2 was "Four doses group"; subjects were vaccinated with 20 μg of HBV vaccine on day 0, 28, months 2 and 6. Group 3, "Four double doses group" were vaccinated with 40 μg of HBV vaccine on day 0, 28, months 2 and 6. Healthy individuals were vaccinated with the standard regimen similar to HIV+ participants in group 1. The vaccine was injected into the deltoid muscle on participants' preferential arm except in four double doses group in which the vaccines were injected on both arms. Twenty-two millilitres of venous blood were collected prior to each vaccination at day 0 (D0) as baseline, day 7 (D7), day 28 (D28), month 2 (2m) (only for the four doses and four double doses groups), month 6 (6m), month 7 (7m), and month 12 (12m).

Table 5.1 Vaccination schedule of recombinant HBsAg vaccine in this study

	Dose of recombinant HBs antigen			
	D0	**D28**	**Month 2**	**Month 6**
HIV+ individuals				
Standrard dose group	20 μg	20 μg		20 μg
Four doses group	20 μg	20 μg	20 μg	20 μg
Four double doses group	40 μg	40 μg	40 μg	40 μg
Healthy control				
Standrard dose	20 μg	20 μg		20 μg

5.2.2. Study population

Participants in this study were the same subjects as the study on comparison of immunogenicity and safety of different regimens of hepatitis B vaccination in HIV-infected adults [228]. Over eighteen years old of 132 HIV+ and 40 HIV– healthy individuals who were seronegative for hepatitis B surface antigen (HBsAg), antibody to hepatitis B surface antigen (anti-HBs), and antibody to hepatitis B core antigen (anti-HBc), antibody to hepatitis C virus (anti-HCV) and without a history of previous HBV vaccination, were enrolled at Maharaj Nakorn Chiang Mai hospital between February 2011 to May 2012. HIV+ participants were eligible to participate if they had absolute number of CD4+ T cells more than 200 cells/mm^3, HIV viral load less than 50 copies/mL and received ART. The exclusion criteria included being pregnant or breast-feeding, having history of hypersensitivity to any component of the vaccine or other immunocompromised conditions besides HIV (e.g., solid organ transplant) or renal insufficiency (creatinine clearance < 30 mL/min), decompensated cirrhosis (Child-Pugh class C), receiving chemotherapy or radiation for active malignancy treatment or immunosuppressive (e.g., corticosteroid ≥ 0·5 mg/kg/day) or immunomodulating treatment in the last six months before screening visit. At month 7 after vaccination, the percentages of sero-responders (anti-HBs ≥ 10 mIU/mL) were 88.6% in the HIV+ Standard doses group, 93.2% in the HIV+ four doses group, 95.4% in the HIV+ four double doses group and 94.7% in HIV– healthy control group. Demographic and clinical characteristics have been reported previously [228], and is summarised in

table 5.2. Healthy controls were confirmed HIV negative by using a rapid immunochromatographic screening assay (Pacific Biotech, Bangkok,Thailand).

5.2.3. Isolation of PBMCs by gradient centrifugation

As described in chapter 2 section 2.3

5.2.4. Cell stimulation

Cryopreserved PBMCs were thawed and rested overnight as described in Chapter 2, section 2.4. Recovered PBMCs were stimulated with complete RPMI containing 5 μg/ml of PHA, 2 μg/ml of recombinant HBsAg (Mybiosource, San Diego, CA, USA) or complete media (unstimulated control). Cells were cultured in 37°C 5% CO_2 incubator for 16-18 hr. Cytokine-producing and CD107a-expressing CD8+ T cells were assessed by ICS assay.

5.2.5. Determination of cytokine-secreting T cells in response to specific antigen by ICS technique

As described in chapter 2 section 2.5

Table 5.2 Baseline demographics and clinical characteristics of participants by vaccination regimen.

Characteristics	HIV+			HIV- (n=38)
	Standard doses (n=44)	4 doses (n=44)	4 double doses (n=44)	
Female	36 (81.8%)	35 (79.6%)	25 (56.8%)	33 (86.8%)
Age (years)	41.0 ± 6.3	42.2 ± 7.6	41.0 ± 6.2	33.2 ± 9.5
CD4+ cell count	400 (314, 558)	554 (416, 731)	544 (410, 642)	ND
CD4+ cell count by category				
CD4+ 201-350 cell/mm^3	14 (31.8%)	5 (11.4)	5 (11.4)	ND
CD4+ > 350 cell/mm^3	30 (68.2%)	39 (88.6)	39 (88.6)	ND
Nadir CD4+ cell count	70 (31,143)	70 (33, 179)	90 (40, 173)	ND
Time elapsed since HIV diansis (months)	134 (79, 182)	98 (83, 164)	120 (92,170)	NA
Current cART				
NNRTI based	39 (88.6%)	39 (88.6%)	40 (90.9%)	NA
PI based	4 (9.1%)	4 (9.1%)	4 (9.1%)	NA
Others	1 (2.3%)	1 (2.3%)	0 (0%)	NA
Duration of cART (months)	80 (47, 90)	86 (64, 99)	92 (75, 110)	NA
Duration of suppressed plasma HIV-1 RNA (months)	37.3 (27.2, 71.3)	73.2 (32.8,76.8)	72.2 (34.2, 77.0)	NA
History of drug resistance	3 (6.8%)	4 (9.1%)	2 (4.5%)	NA
Underlying diseases				
Diabetes mellitus and IFG[a]	3 (6.8%)	5 (11.4%)	8 (18.1%)	ND
Hypertension	3 (6.8%)	8 (18.2%)	10 (22.7%)	ND
Dyslipidemia	7 (15.9%)	6 (13.6%)	7 (15.9%)	ND
Others	5 (11.4%)	8 (18.2%)	8 (18.2%)	ND
Number of responder at month 7	39 (88.6%)	41 (93.2%)	42 (95.4%)	36 (94.7%)

Data presented in number (%), means ± SD, or median (IQR). Abbreviation: ND, not done; NA, not applicable; cART, combination antiretroviral therapy; NNRTI, non-nucleoside/nucleotide reverse transcriptase inhibitor; PI, protease inhibitor; IFG, impaired fasting glucose

[a] Defined as fasting plasma glucose from 5.6 mmol/L (100 mg/dL) to 6.9 mmol/L (125 mg/dL)

5.3 Results

5.3.1. Cytokine production and CD107a expression of CD4 and CD8 T cells

Flow cytometric analysis of cytokine-producing and CD107a-expressing CD4+ or CD8+ T cells were performed as described in chapter 2 section 2.6 (Fig. 2.1A). Results are presented as fold increases of the percentages of TNF-α, IFN-γ, IL-2 and IL-10 production or expression of CD107a in response to *in vitro* recombinant HBsAg stimulation over medium alone. The fold increases of the percentages of cytokine-producing and CD107a-expressing CD4+ and CD8+ T cells in each study groups were compared between D0 as baseline, D7, 1m, 2m (only for the four doses and four double doses groups), 6m, 7m and 12m after vaccination with Hepavax-Gene vaccine. In healthy control group, only the median fold increase of TNF-α-producing CD4+ T cells in response to recombinant HBsAg stimulation *in vitro* at 7m was statistically significantly higher than D0 ($p = 0.009$), D7 ($p = 0.002$), 6m($p = 0.024$) and 12m ($p = 0.027$) but not at 1m ($p = 0.075$) after vaccination (Figure 5.1A). No differences in the median fold increase of TNF-α-producing CD8+ T cells (Figure 5.1C) or IFN-γ (Figure 5.1B and G), IL-2 (Figure 5.1C and H) and IL-10 (Figure 5.1D and I)-producing and CD107a-expressing CD4+ (Figure 5.1 E) and CD8+ T cells (Figure 5.1 J) between all-time points of study were observed in this study group. There were no statistical differences of the median fold increases of cytokine-producing and CD107a-expressing CD4+ and CD8+ T cells between all-time points in all three HIV+ study groups (Figure 5.2-5.4).

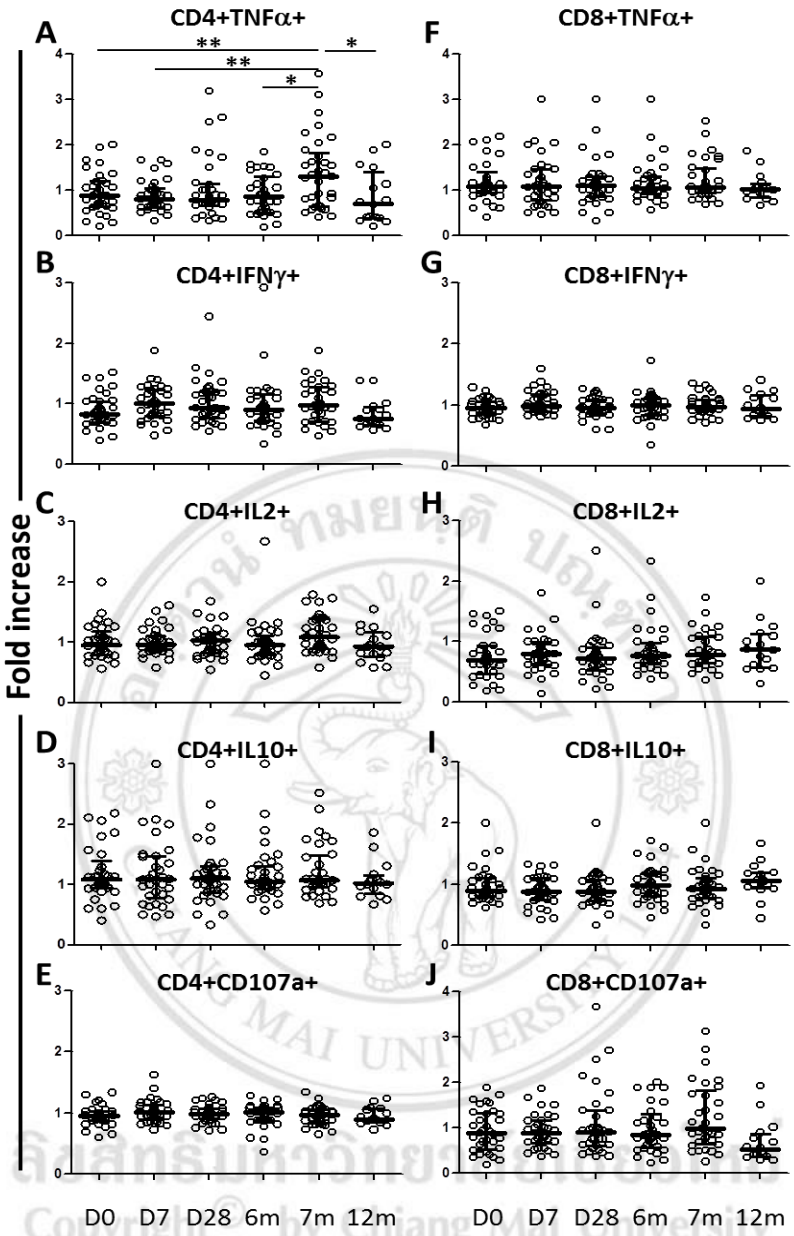

Figure 5.1. Fold increases of cytokine-producing and CD107a-expressing T cells in healthy control individuals. Fold increases of TNF-α, IFN-γ, IL-2, and IL-10 production, and CD107a–expression, by CD4+ (A-E) and CD8+ (F-J) T cells in response to recombinant HBsAg by HIV– individuals who were vaccinated with standard dose regimen, compared before *in vivo* vaccination on day zero (D0), and at days 7 (D7), day 28 (D28), 6 month (6M), 7 month (7M) and 12 months (12M) after vaccination. Medians represented by thick, wide horizontal bars, and 25%-75% interquartile ranges by thin, narrow bars. *p*-value <0.05 were considered statistically significant. * and ** show $p < 0.05$ and 0.01, respectively.

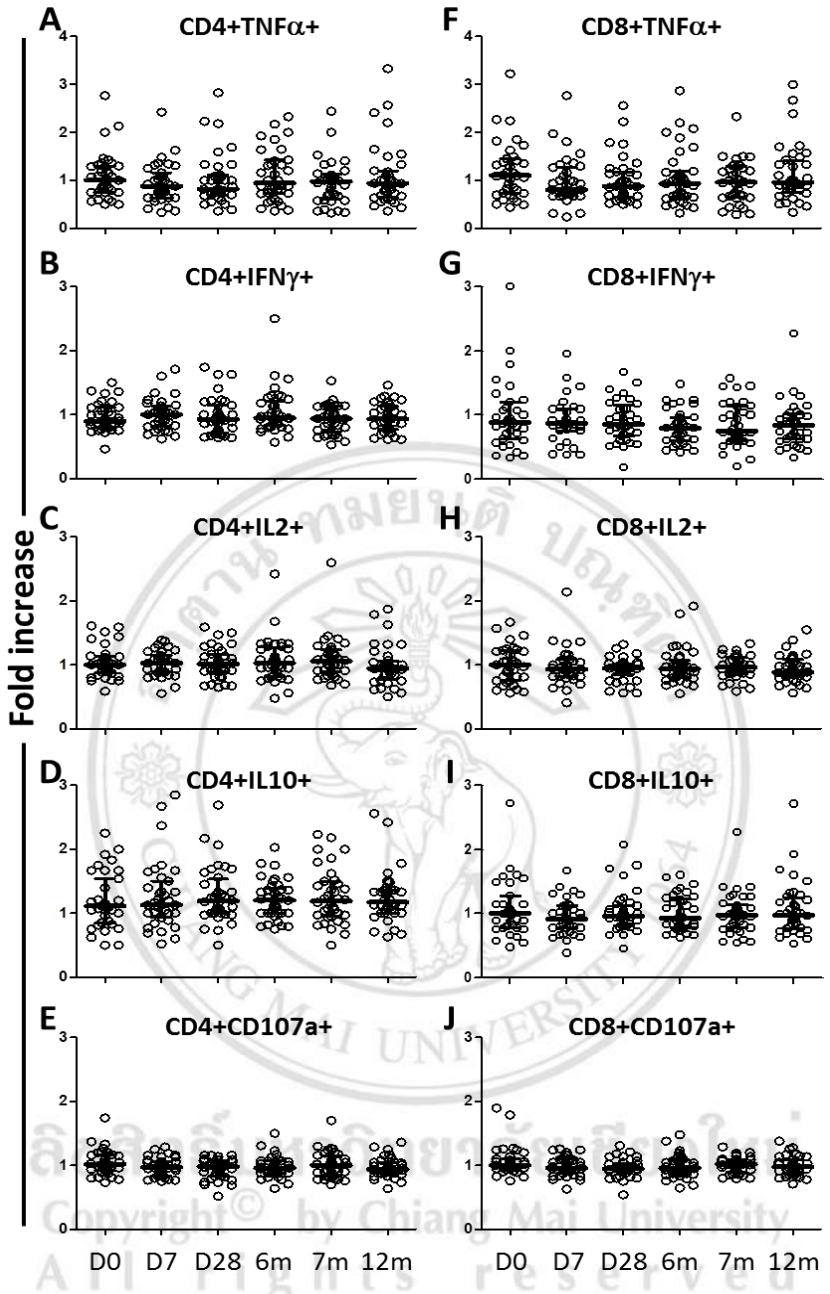

Figure 5.2. Fold increases of cytokine-producing and CD107a-expressing T cells in the standard dose group of HIV+ individuals. Fold increases of TNF-α, IFN-γ, IL-2, and IL-10 production, and CD107a–expression, by CD4+ (A-E) and CD8+ (F-J) T cells in response to recombinant HBsAg by HIV+ individuals who were vaccinated with the standard dose regimen, compared before *in vivo* vaccination on day zero (D0), and at days 7 (D7), day 28 (D28), 6 month (6M), 7 month (7M) and 12 months (12M) after vaccination. Medians represented by thick, wide horizontal bars, and 25%-75% interquartile ranges by thin, narrow bars.

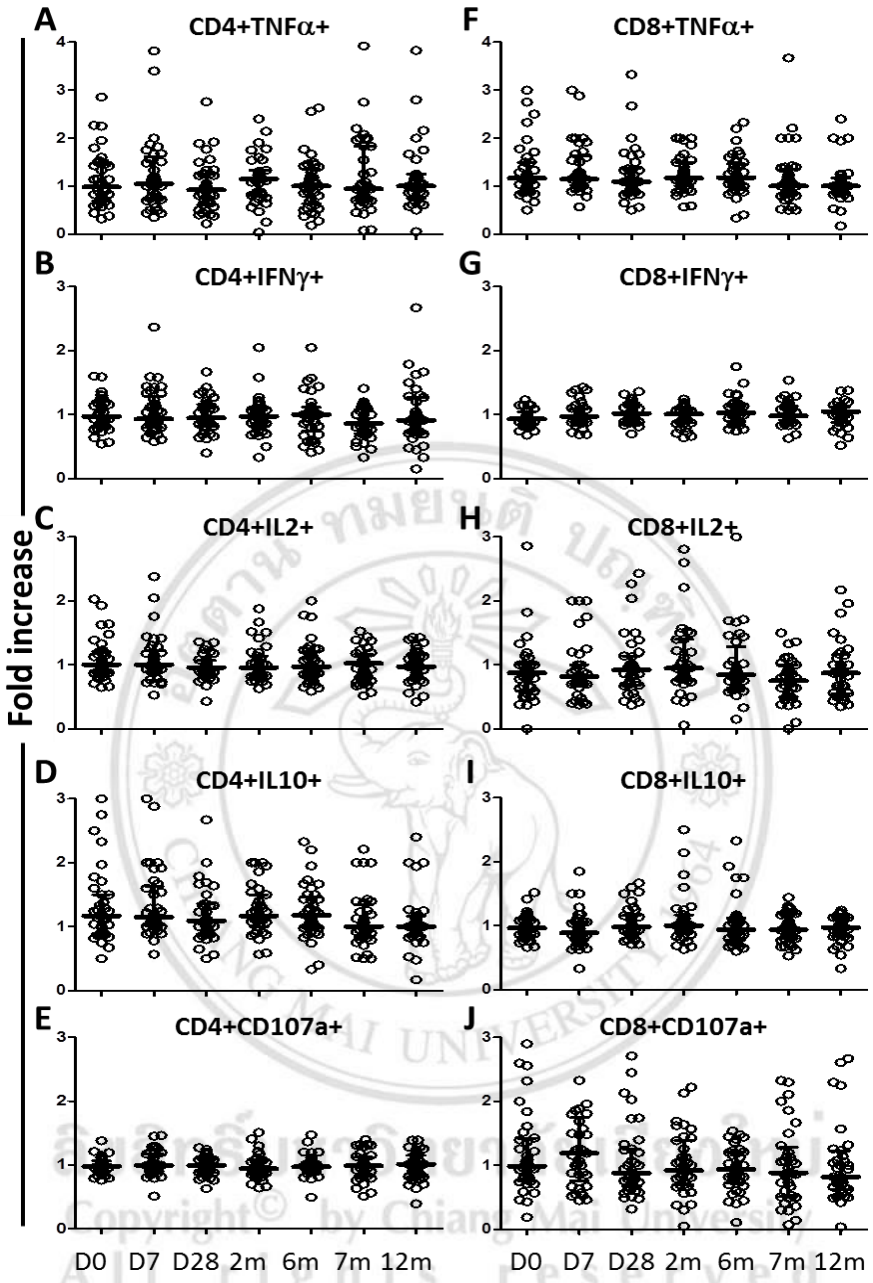

Figure 5.3. Fold increases of cytokine-producing and CD107a-expressing T cells in the four doses group of HIV+ individuals. Fold increases of TNF-α, IFN-γ, IL-2, and IL-10 production, and CD107a–expression, by CD4+ (A-E) and CD8+ (F-J) T cells in response to recombinant HBsAg by HIV+ individuals who were vaccinated with the four doses regimen, compared before *in vivo* vaccination on day zero (D0), and at days 7 (D7), day 28 (D28), 6 month (6M), 7 month (7M) and 12 months (12M) after vaccination. Medians represented by thick, wide horizontal bars, and 25%-75% interquartile ranges by thin, narrow bars.

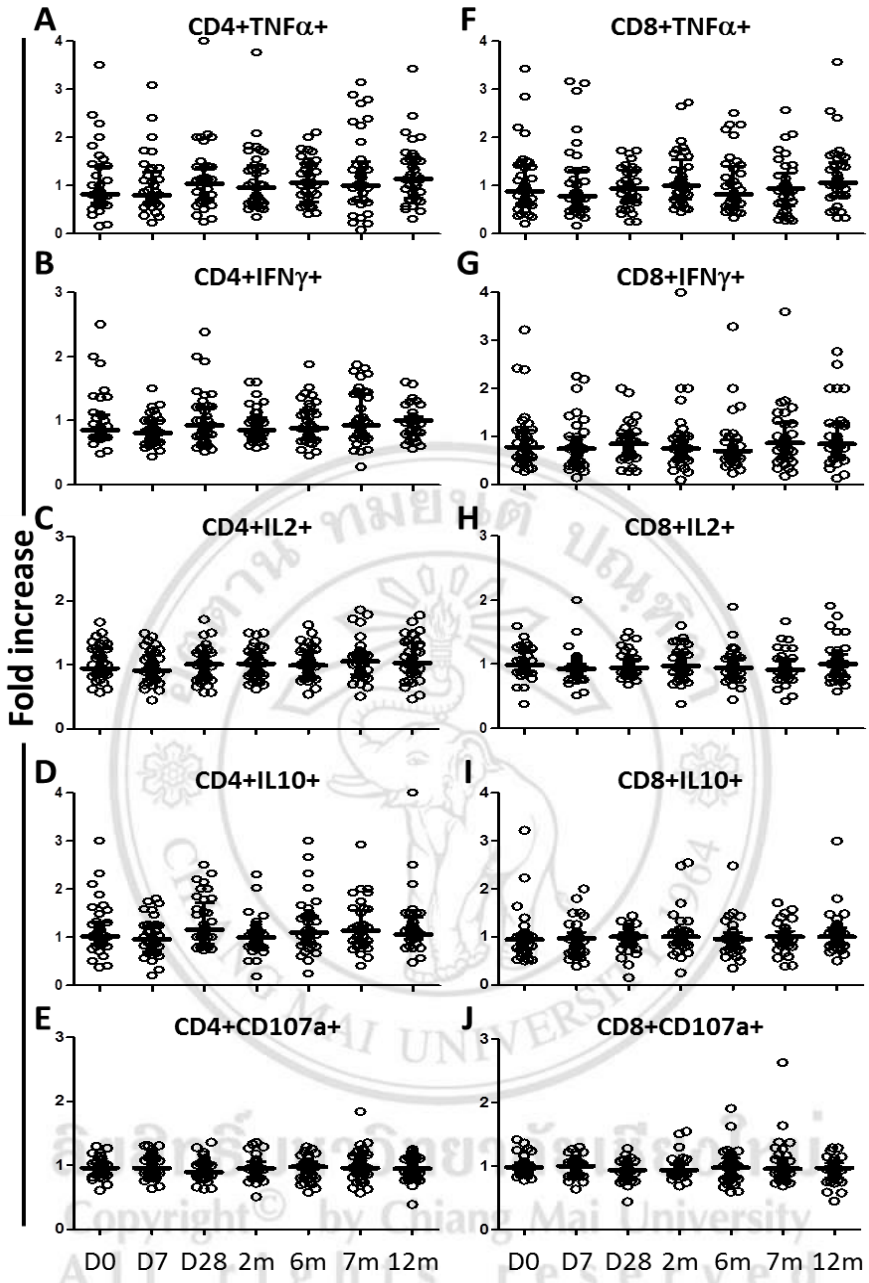

Figure 5.4. Fold increases of cytokine-producing and CD107a-expressing T cells in the four double doses group of HIV+ individuals. Fold increases of TNF-α, IFN-γ, IL-2, and IL-10 production, and CD107a–expression, by CD4+ (A-E) and CD8+ (F-J) T cells in response to recombinant HBsAg by HIV+ individuals who were vaccinated with the four double doses regimen, compared before *in vivo* vaccination on day zero (D0), and at days 7 (D7), day 28 (D28), 6 month (6M), 7 month (7M) and 12 months (12M) after vaccination. Medians represented by thick, wide horizontal bars, and 25%-75% interquartile ranges by thin, narrow bars.

Data were further analysed by comparing the median fold increases between study groups. Anova analysis of cytokine-producing and CD107a-expressing CD4+ or CD8+ T cells did not reveal statistically significant differences between groups at D0 (Figure 5.5). No statistical differences in the median fold increases of TNF-α-producing CD8+ T cells (Figure 5.5 F) and IFN-γ (Figure 5.5 B and G), IL-2 (Figure 5.5 C and H) and IL-10 (Figure 5.5 D and I)-producing and CD107a-expressing CD4+ and CD8+ T cells (Figure 5.5 E and J) between groups at 7m were observed. However, the median fold increase of TNF-α-producing CD4+ T cells (Figure 5.5 A) in response to recombinant HBsAg stimulation *in vitro* at 7m of healthy control group was statistically significantly higher than the standard dose HIV+ group ($p = 0.048$), but not different from the four doses group ($p = 0.407$) or the four double doses group ($p = 0.497$). When evaluated with regard to serological responsiveness to the vaccine (seropositive [n = 96] versus seronegative [n = 11]) among HIV+ individuals at 7m, Anova analysis of cytokine-producing and CD107a-expressing CD4+ or CD8+ T cells did not reveal statistically significant differences (Fig 5.6A-J).

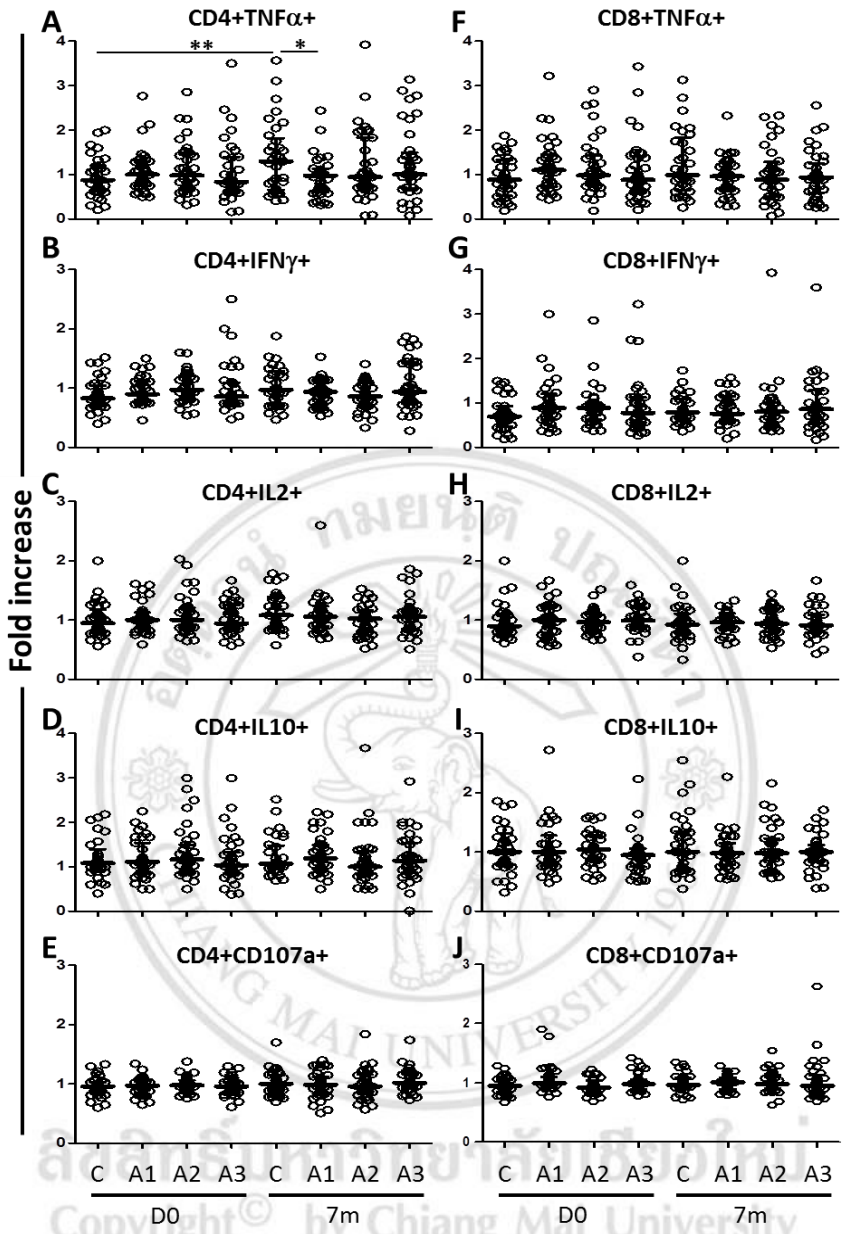

Figure 5.5. Fold increases of cytokine-producing and CD107a-expressing T cells between study groups. Fold increases of TNF-α, IFN-γ, IL-2, and IL-10 production, and CD107a–expression, by CD4+ (A-E) and CD8+ (F-J) T cells in response to recombinant HBsAg between healthy controls (C), the standard dose group (A1), the four doses group (A2) , and the four double doses group (A3) were compared before *in vivo* vaccination on day zero (D0) and 7 month (7M) after vaccination. Medians represented by thick, wide horizontal bars, and 25%-75% interquartile ranges by thin, narrow bars. *p*-value <0.05 were considered statistically significant. * and ** show $p < 0.05$ and 0.01, respectively.

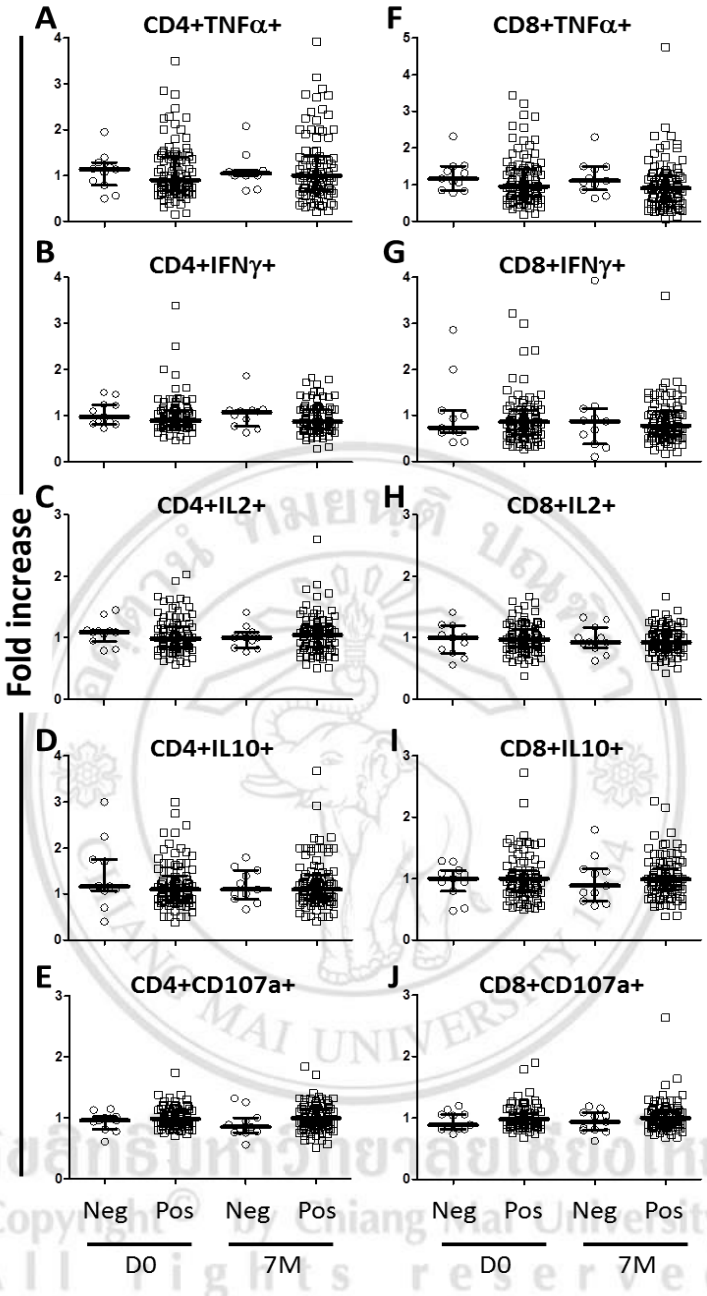

Figure 5.6. Fold increases of cytokine-producing and CD107a-expressing T cells between groups with regards to serological responsiveness to the vaccine. Fold increases of TNF-α, IFN-γ, IL-2, and IL-10 production, and CD107a–expression, by CD4+ (A-E) and CD8+ (F-J) T cells in response to recombinant HBsAg between seronegative (Neg) and seropositive (Pos) individuals among all HIV+ participants were compared before *in vivo* vaccination on day zero (D0) and 7 month (7M) after vaccination. Medians represented by thick, wide horizontal bars, and 25%-75% interquartile ranges by thin, narrow bars.

5.3.2. Cytokine production and CD107a expression of CD4 and CD8 memory T cells

Gating strategies of cytokine-producing and CD107a-expressing CD4+ or CD8+ CD45RO+ memory T cells were established as described in chapter 2 section 2.6 (Fig. 2.2). The fold increases of TNF-α, IFN-γ, IL-2, and IL-10-producing and CD107a-expressing memory CD4+ or CD8+ T cells in response to *in vitro* recombination HBsAg stimulation over medium alone were compared between time points of study in each group. In healthy control group, there were no statistical differences of IFN-γ (Figure 5.7 B and G) and IL-10 (Figure 5.7 D and I)-producing or CD107a-expressing (Figure 5.7 E and J) memory CD4+ or CD8+ T cells between all-time points of study. However, the fold increases of TNF-α-producing memory CD4+ T cells (Figure 5.7 A) at 7m after vaccination were significantly higher than that at 6m (p = 0.042) and 12m (p = 0.022), but not at D0 (p = 0.067), D7 (p = 0.09) and 1m (p = 0.116). The fold increases of IL-2-producing memory CD4+ T cells (Figure 5.7 C) at 7m after vaccination were statistically significant higher than that at D0 (p = 0.008), D7(p = 0.004), 1m (p = 0.006) and 6m (p = 0.014) but not at 12m (p = 0.052). In contrast to memory CD4+ T cells, no differences in the fold increase of cytokine production and expression of CD107a of memory CD8+ T cells were observed at all-time points of study. In all study groups of HIV+ individuals, there were no statistical differences of the median fold increases of cytokine production and expression of CD107a of memory CD4+ and CD8+ T cells in response to *in vitro* recombinant HBsAg stimulation at all-time points of study (Figure 5.8-5.10).

When compared between 4 groups at D0 and 7m, Anova statistical analysis of the fold increases of cytokine-producing and CD107a-expressing memory CD4+ and CD8+ T cells did not demonstrate statistically significant differences between groups (Figure 5.11). There were no statistical differences of the median fold increases of all cytokine-production and expression of CD107a memory CD4+ and CD8+ T cells between HBV vaccine serological non-responders and responders at any time point of the study (data not shown).

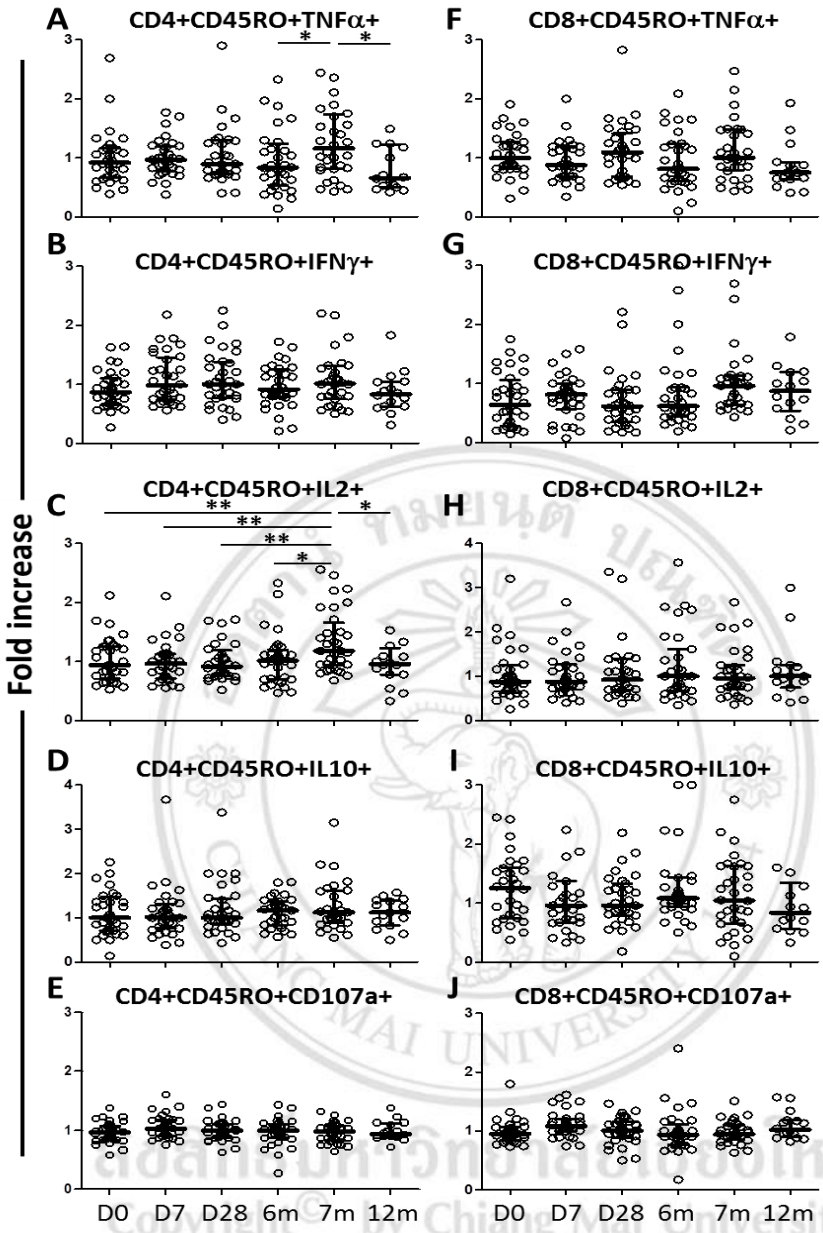

Figure 5.7. Fold increases of cytokine-producing and CD107a-expressing memory T cells in HIV– individuals. Fold increases of TNF-α, IFN-γ, IL-2, and IL-10 cytokine production, and CD107a–expression, by memory CD4+ (A-E) and CD8+ (F-J) T cells in response to recombinant HBsAg by HIV– individuals who were vaccinated with the standard dose regimen, compared before *in vivo* vaccination on day zero (D0), and at days 7 (D7), day 28 (D28), 6 month (6M), 7 month (7M) and 12 months (12M) after vaccination. Medians represented by thick, wide horizontal bars, and 25%-75% interquartile ranges by thin, narrow bars. *p*-value <0.05 were considered statistically significant. * and ** show $p < 0.05$ and 0.01, respectively.

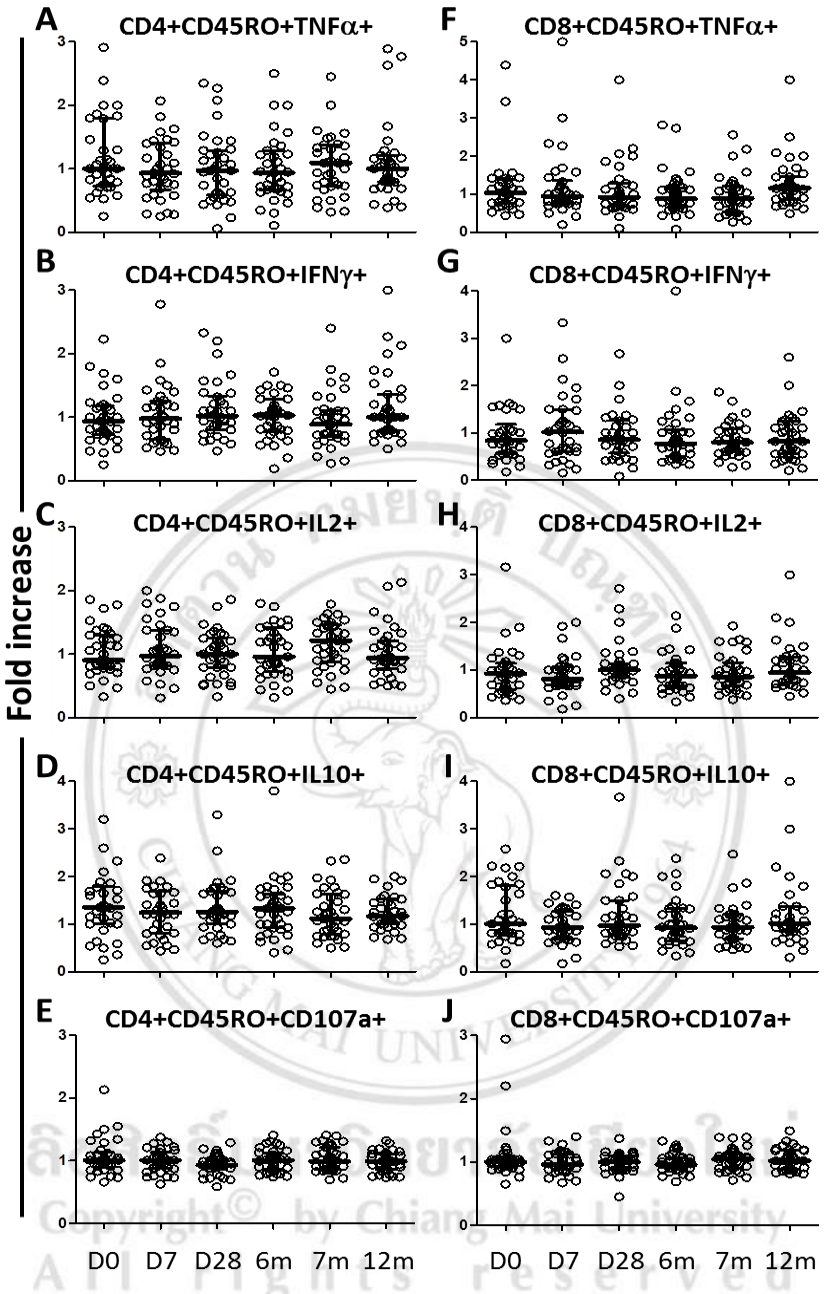

Figure 5.8. Fold increases of cytokine-producing and CD107a-expressing memory T cells in the standard dose group of HIV+ individuals. Fold increases of TNF-α, IFN-γ, IL-2, and IL-10 cytokine production, and CD107a–expression, by memory CD4+ (A-E) and CD8+ (F-J) T cells in response to recombinant HBsAg by HIV+ individuals who were vaccinated with the standard dose regimen, compared before *in vivo* vaccination on day zero (D0), and at days 7 (D7), day 28 (D28), 6 month (6M), 7 month (7M) and 12 months (12M) after vaccination. Medians represented by thick, wide horizontal bars, and 25%-75% interquartile ranges by thin, narrow bars.

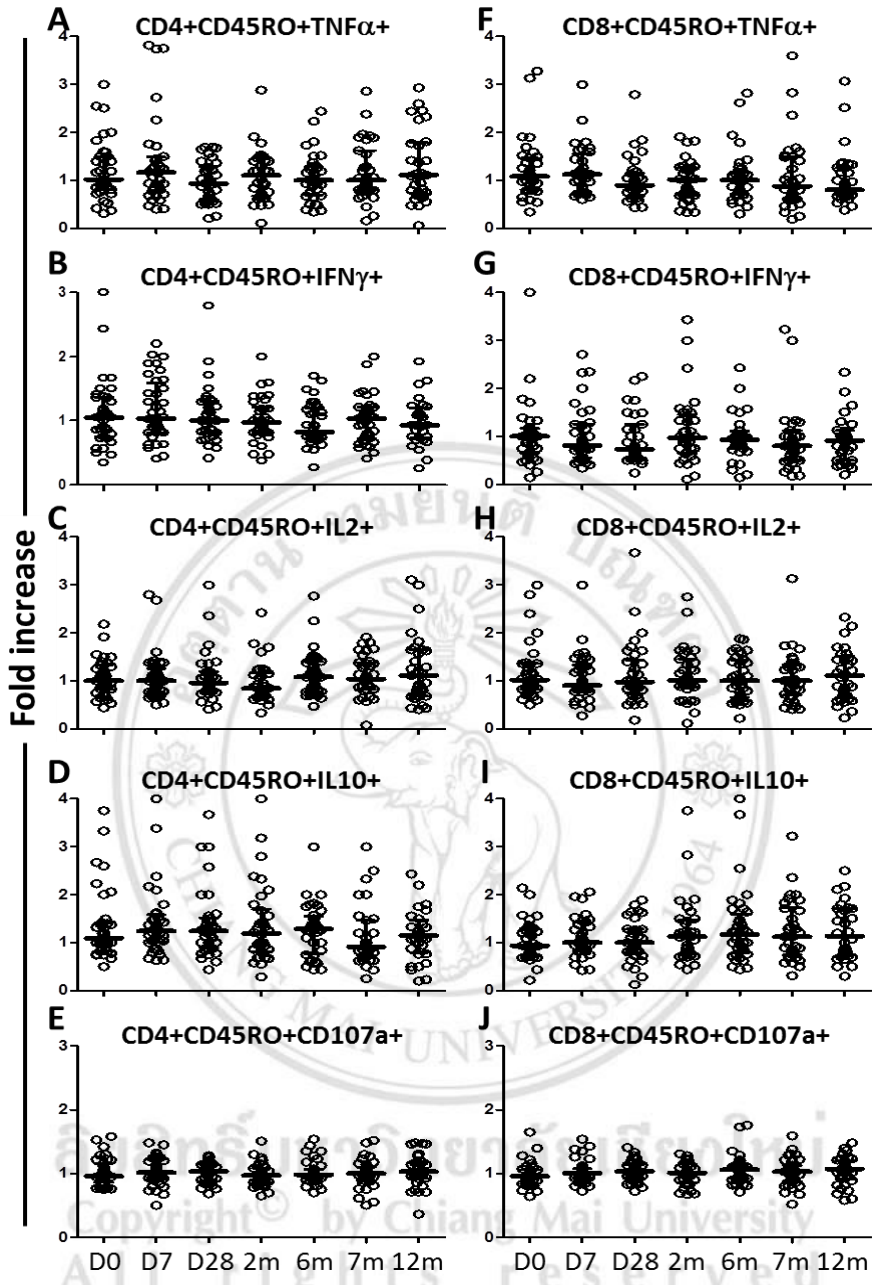

Figure 5.9. Fold increases of cytokine-producing and CD107a-expressing memory T cells in the four doses group of HIV+ individuals. Fold increases of TNF-α, IFN-γ, IL-2, and IL-10 cytokine production, and CD107a–expression, by memory CD4+ (A-E) and CD8+ (F-J) T cells in response to recombinant HBsAg by HIV+ individuals who were vaccinated with the four doses regimen, compared before *in vivo* vaccination on day zero (D0), and at days 7 (D7), day 28 (D28), 6 month (6M), 7 month (7M) and 12 months (12M) after vaccination. Medians represented by thick, wide horizontal bars, and 25%-75% interquartile ranges by thin, narrow bars.

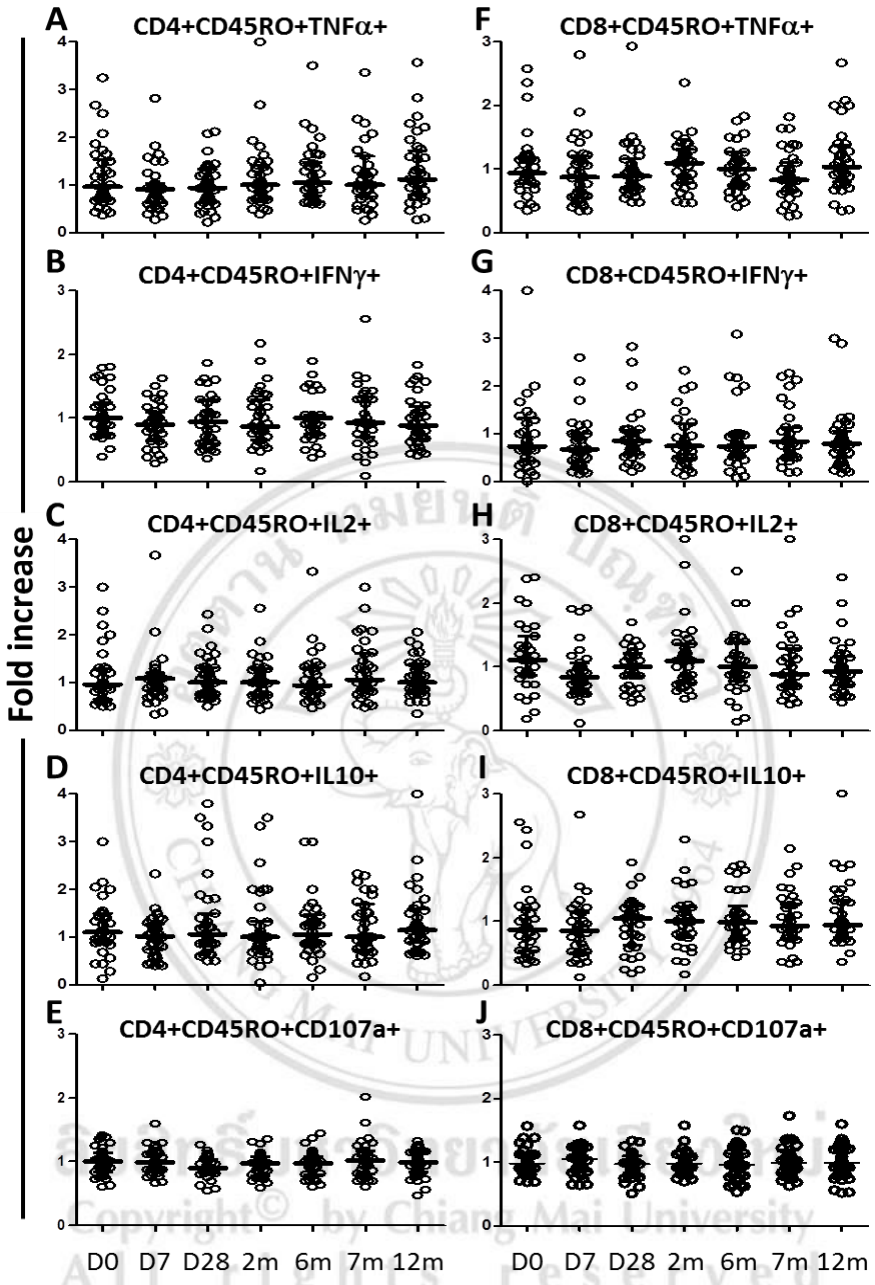

Figure 5.10. Fold increases of cytokine-producing and CD107a-expressing memory T cells in the four double doses group of HIV+ individuals. Fold increases of TNF-α, IFN-γ, IL-2, and IL-10 cytokine production, and CD107a–expression, by memory CD4+ (A-E) and CD8+ (F-J) T cells in response to recombinant HBsAg, by HIV+ individuals who were vaccinated with the four double doses regimen, compared before *in vivo* vaccination on day zero (D0), and at days 7 (D7), day 28 (D28), 6 month (6M), 7 month (7M) and 12 months (12M) after vaccination. Medians represented by thick, wide horizontal bars, and 25%-75% interquartile ranges by thin, narrow bars.

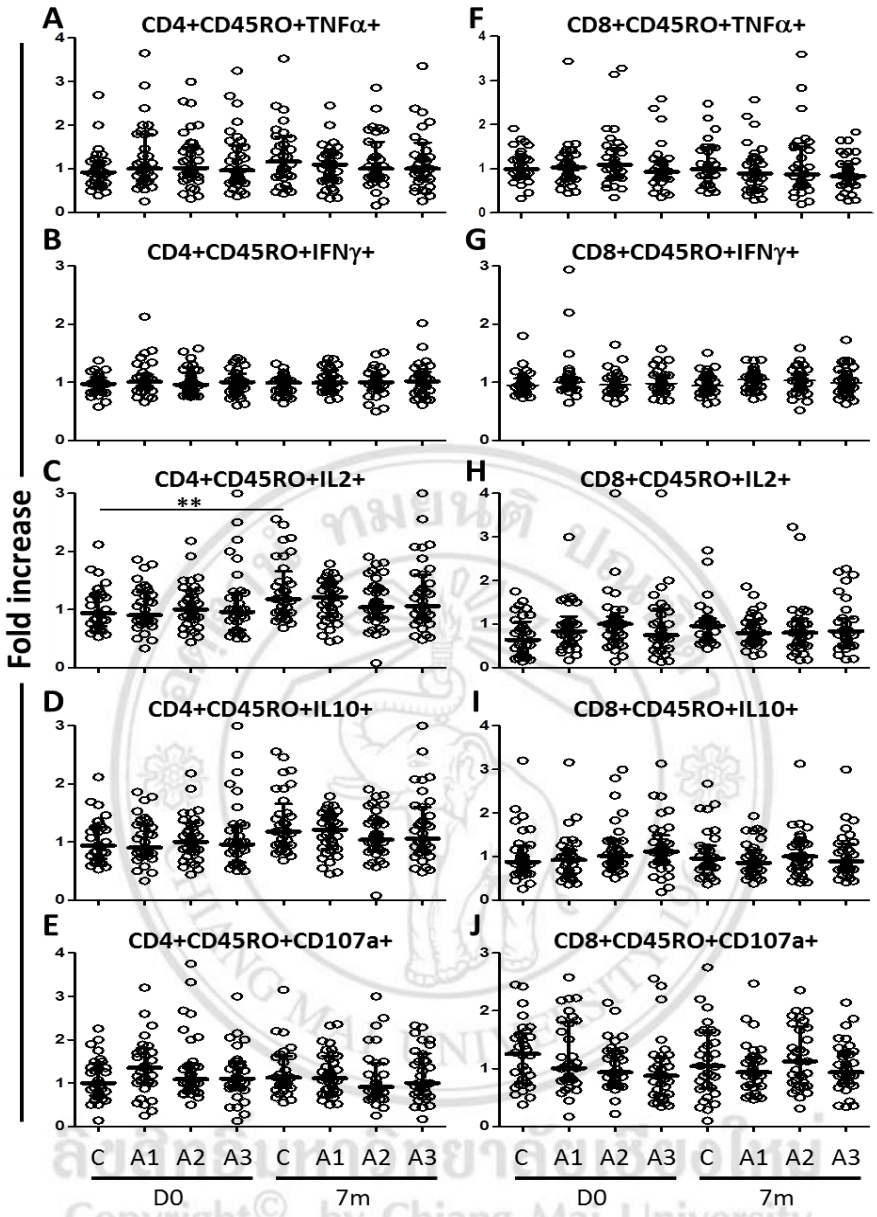

Figure 5.11. Fold increases of cytokine-producing and CD107a-expressing memory T cells between study groups. Fold increases of TNF-α, IFN-γ, IL-2, and IL-10 cytokine production, and CD107a–expression, by memory CD4+ (A-E) and CD8+ (F-J) T cells in response to recombinant HBsAg between healthy controls (C), the standard dose group (A1), the four doses group (A2) , and the four double doses group (A3) were compared before *in vivo* vaccination on day zero (D0) and 7 month (7M) after vaccination. Medians represented by thick, wide horizontal bars, and 25%-75% interquartile ranges by thin, narrow bars. *p*-value <0.05 were considered statistically significant. ** show $p < 0.01$.

5.4 Discussion

Owing to HBV share routes of transmission with HIV, infection with HBV in HIV+ population is more frequent than healthy population. The effectiveness of HBV vaccination in HIV+ individuals has been broadly investigated in relation to humoral immune response. In contrast, the knowledge of cellular immune responses is much less well understood. This study focused on cytokine production and expression of degranulation marker of CD4+ and CD8+ T cells in response to *in vitro* recombination HBsAg stimulation in HIV+ individuals compared to healthy donors.

We found no statistical differences in the frequencies of cytokine-producing and CD107a-expressing of total or memory CD4+ or CD8+ T cells during the 12 months of study in all 3 HIV+ groups. However, the results showed the increase of TNF-α-producing total and memory CD4+ T cells at 7m after vaccination in healthy controls compared to HIV+ group whom received the same vaccination regimen (standard dose group). TNF-α has direct effect on the proliferation of HBV-specific CTLs [295]. The up-regulation of TNF-α of HBV-specific CTL abrogates gene expression and replication of HBV without killing infected hepatocytes [148, 296]. TNF-α also plays an important role in the inhibition of HBV-specific regulatory T cells suppressive function [297]. Higher production of TNF-α of HBV-specific CD4+ T cells after vaccination in healthy individuals suggests that, at the standard vaccination regimen, they might achieve better protection from HBV infection compared to HIV+ individuals.

We also found the increase of IL-2-producing memory CD4+ T cells in response to recombinant HBV stimulation *in vitro* at 7m after vaccination in healthy control group. IL-2 has effect on many immune cell types, especially lymphocytes for their differentiation and proliferation [298, 299]. It also plays an important role in the homeostasis of the immune system [298, 299]. IL-2 secretion by memory CD4+T cells is also essential for B cells differentiation into IgG-producing plasma cells [300]. In general, vaccination with protein antigens in humans induces antigen-specific T cells to produce IL-2 more dominantly than other cytokines [301, 302]. The increase of IL-2 production in healthy control group would be beneficial to these individuals against

HBV infection. However, comparison of IL-2-producing memory CD4+ T cells between all four groups at 7m after vaccination, which was the peak of response in healthy controls, did not show statistical difference. In addition, no significant increases of cytokine-producing T cells or memory T cells were observed at all time points during investigation in HIV+ groups. This might be the result of little increases of these HBV-specific cells, or the responses to the four doses and four double doses in HIV+ groups were comparable to the standard dose in healthy controls. It should be noted that the absolute CD4 T cell counts of HIV+ groups in our study and their viral loads were in good levels. All of them were also receiving ART. This could be a reason that no profound differences were observed in this study. Furthermore, the HBV vaccine used in this study was highly immunogenic in our populations as reflected by high seroconversion rate in all three HIV+ study groups [228]. The percentage of serological responders to HBV vaccination in HIV+ adults receiving the standard vaccination regimen was almost as high as that achieved in non-HIV healthy adults, and much higher seroconversion rate than that reported by other studies [211, 225, 289, 303]. Such effective vaccine might induce CMI responses to a nearly comparable level in all groups.

The correlation between cellular and humoral immunity in response to HBV vaccination is still controversial. We did not find any differences of cytokine production between serological responders and non-responders among HIV+ individuals. Our results were similar to that reported in healthy individuals [304]. Nevertheless, the production of cytokines observed in serological non-responders in our and other studies [304] may shed light on the induction of specific cellular immunity to control HBV infection in this population. This issue warrants further investigation.

In conclusion, this study suggests that the standard HBV vaccination schedule induces the TNF-α and IL-2-producing CD4+ T cells at 7m after vaccination in healthy individuals but only TNF-α -producing CD4+ T cells better than HIV+ individuals who received the same vaccination regimen. Although further investigations are needed in order to explore the mechanisms that may contribute to protective CMI, the information from this study may provide valuable perception for future vaccine design to improve cellular immune responses to HBV vaccination for HIV+ populations.