

CHAPTER IV

RESULTS

1. Clinical characteristics

The clinical characteristics were summarized in Table 1. All 20 patients were asymptomatic and antiretroviral naïve. Fourteen were infected with CRF01_AE and 2 patients were infected with subtype B'. The CD4+ T cells were ranged from 303 to 968 cells/ cu.mm. with mean of 503 cells/ cu.mm. The plasma HIV-RNA were ranged from 2,738 to >500,000 copies/ mL with the median of 7,866 copies/ mL (In one patient whose plasma HIV-RNA of >500,000 was treated as 500,000 for calculation purposes)

Table 1: Clinical information of HIV-1 infected patients

Patient No.	Initial	Sex	Age (years)	HIV-1 subtype	CD4+T cells (cells/cu.mm.)	HIV-1 RNA (copies/mL)
1	OK	M	27	B'	327	5,207
2	AO	F	32	CRF01_AE	334	53,684
3	TB	F	27	CRF01_AE	386	6,602
4	PA	F	25	CRF01_AE	968	4,878
5	DK	M	29	CRF01_AE	909	26,309
6	TW	F	45	CRF01_AE	406	10,176
7	PK	F	49	B'	332	49,565
8	AP	F	34	CRF01_AE	321	2,738
9	KK	F	24	CRF01_AE	482	10,268
10	TS	M	45	CRF01_AE	435	14,638
11	KP	F	31	CRF01_AE	809	8,976
12	JN	F	27	ND	377	4,232
13	PC	F	36	CRF01_AE	471	5,269
14	JL	F	34	CRF01_AE	429	16,526
15	CT	M	35	CRF01_AE	638	43,059
16	CY	F	32	CRF01_AE	303	>500,000
17	ST	F	35	CRF01_AE	487	4,954
18	RM	F	32	ND	454	3,761
19	PN	F	35	ND	564	6,755
20	HN	M	20	ND	636	3,779

M = male, F = female
 ND = not done

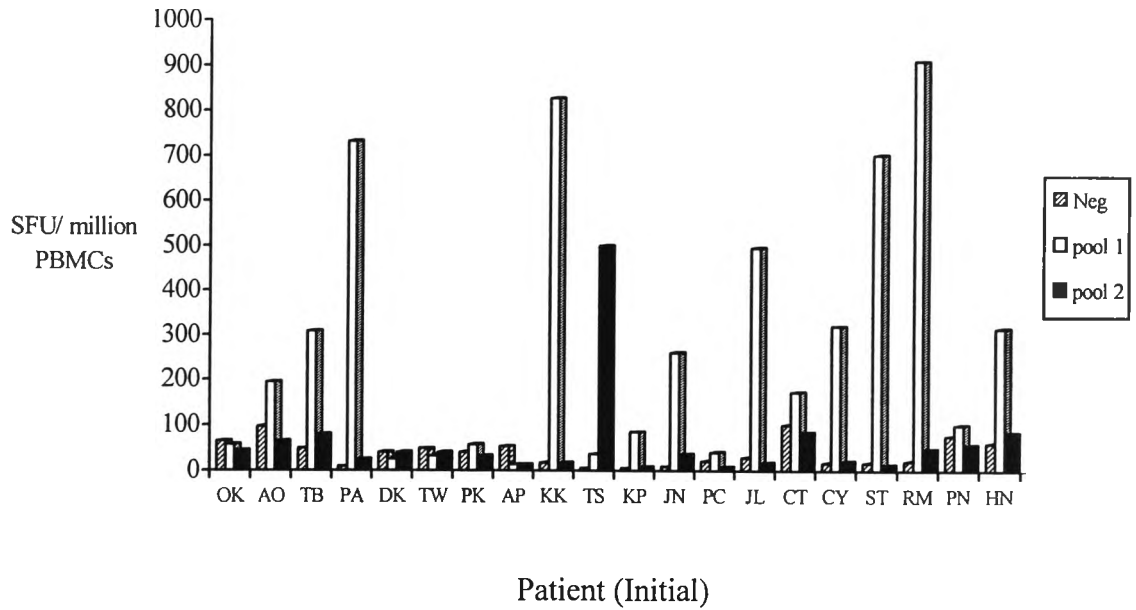
2. HIV-1 CRF01_AE-specific Elispot-based CTL responses against pooled Tat peptides

As shown in table 2, 10 patients (50%) showed IFN- γ -Elispot responses to Tat pooled peptides (Fig.4). The magnitude of responses ranged from 260 to 912 SFU/10⁶ PBMCs. (Median, 498 SFU/10⁶ PBMCs; mean, 538 SFU/10⁶ PBMCs)

Nine patients (45%) showed IFN- γ -Elispot responses to Tat peptide pool 1 (Fig 6). The magnitude of responses ranged from 260 to 912 SFU/10⁶ PBMCs. (Median, 496 SFU/10⁶ PBMCs; mean, 542 SFU/10⁶ PBMCs). There is only one patients (5%) showed IFN- γ -Elispot response to Tat peptide pool 2 (Fig 7). The magnitude of response was 500 SFU/10⁶ PBMCs)

Table 2: IFN- γ -Elispot responses to pooled Tat peptides in 20 patients

Patient No.	Initial	IFN- γ -Elispot response (SFU/10 ⁶ PBMCs)		
		Negative	Pool 1	Pool 2
1	OK	64	56	44
2	AO	96	194	64
3	TB	48	308	80
4	PA	8	732	24
5	DK	40	24	40
6	TW	48	32	40
7	PK	40	56	32
8	AP	52	12	12
9	KK	16	828	16
10	TS	4	36	500
11	KP	4	84	8
12	JN	8	260	36
13	PC	20	40	8
14	JL	28	496	16
15	CT	100	172	84
16	CY	16	320	20
17	ST	16	704	12
18	RM	20	912	48
19	PN	76	100	56
20	HN	60	316	84

Figure 4: IFN- γ -Elispot responses to pooled Tat peptides in 20 patients

20 patients (x -axis) were screened in the IFN- γ -Elispot assay using 2 pools of HIV-1 CRF_AE based, 17-21 mer synthetic truncated Tat peptides overlapping by 10 amino acids. The responses were expressed as SFU/ 10^6 PBMCs (y -axis)

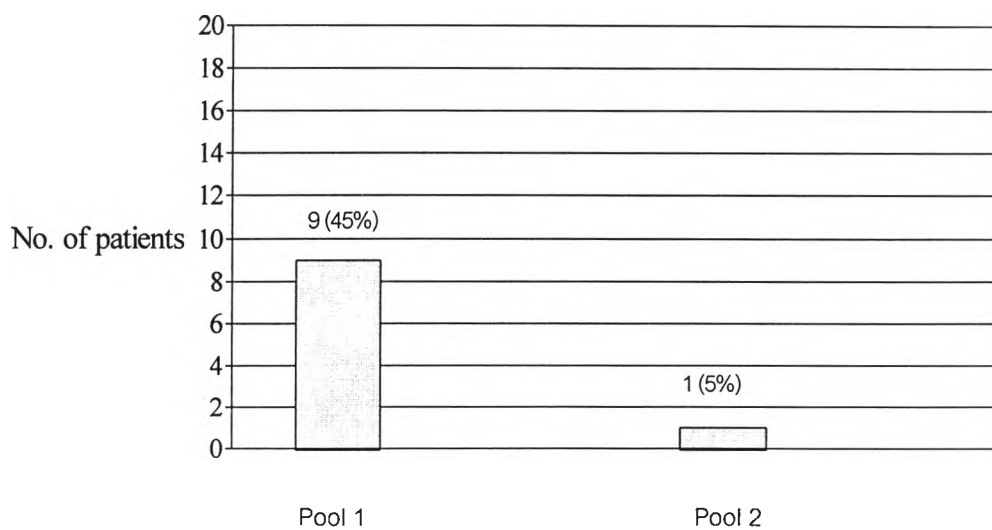
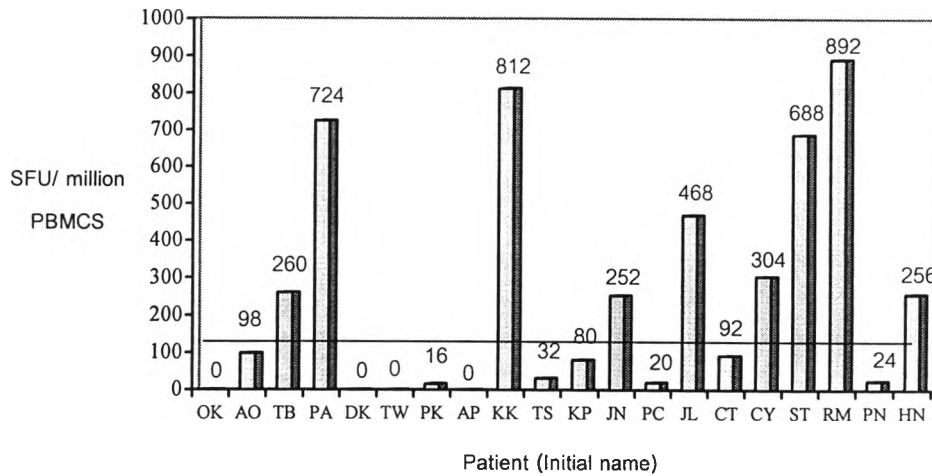
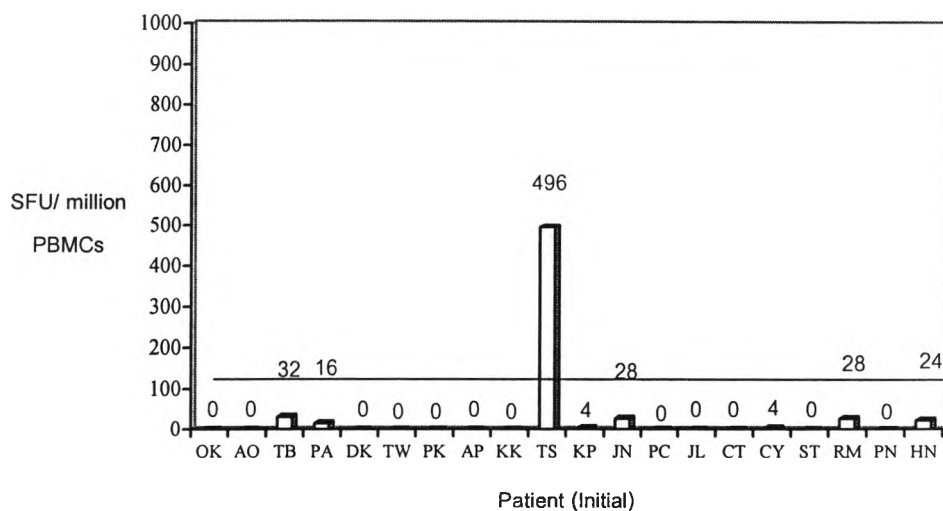
Figure 5: IFN- γ -Elispot responses to pooled Tat peptides

Figure 6: IFN- γ -Elispot responses to Tat peptides pool 1
(Background subtracted)



9 patients showed IFN- γ -Elispot responses to Tat peptide pool 1. The responses was considered as a positive result if the number of established SFU/ 10^6 PBMCs subtracted by negative control is ≥ 100 and 2 folds more than background (In patients whose the number that of SFU after subtracted by negative control is equal or less than 0 are shown as 0 in this graph)

Figure 7: IFN- γ -Elispot responses to Tat peptides pool 2
(Background subtracted)



Only 1 patient showed IFN- γ -Elispot responses to Tat peptide pool 2. Responses were considered as a positive result if the number of established SFU/ 10^6 PBMCs subtracted by negative control is ≥ 100 2 folds more than background (In patients whose the number of that of SFU after subtracted by negative control is equal or less than 0 are shown as 0 in this graph)

3. HIV-1 CRF_{AE}-specific Elispot –based CTL responses against Tat individual peptides

Seven patients (35%) showed IFN- γ -Elispot responses to individual Tat peptides. The identified IFN- γ -Elispot responses were shown in table 3. There are 7 out of 10 (70%) patients who have responses to pooled peptides showed responses to individual peptides. The magnitude of responses ranged from 128 to 1,264 SFU/10⁶ PBMCs (median, 460 SFU/10⁶ PBMCs; mean, 493 SFU/10⁶ PBMCs)

As shown in figure 8, there is only one (1/20, 5%) patient showed IFN- γ -Elispot response to Tat 1 (Tat 1-21; MELVDPNLEPWNHPGSQPTTA). The magnitude of response was 1,264 SFU/10⁶ PBMCs. Two (2/20, 10%) patients showed IFN- γ -Elispot responses to Tat 2 (Tat 12-29; NHPGSQPTTACSKCYCKK) with the magnitude of responses were 204 and 308 SFU/10⁶ PBMCs. Three (3/20, 15%) patients showed IFN- γ -Elispot responses to Tat 3 (Tat 20-39; TACSKCYCKKCCWHCQLCFL). The magnitude of responses ranged from 240 to 824 SFU/10⁶ PBMCs (median, 324 SFU/10⁶ PBMCs). Six (6/20, 30%) patients showed IFN- γ -Elispot responses to Tat 4 (Tat 30-49; CCWHCQLCFLKKGLGISYGR) with the magnitude of responses ranged from 176 to 704 SFU/10⁶ PBMCs (median, 538 SFU/10⁶ PBMCs). One (1/20, 5%) patient showed IFN- γ -Elispot response to Tat 5 (Tat 40-56; KKGLGISYGRKKRKHRR) (128 SFU/10⁶ PBMCs), and one patient showed IFN- γ -Elispot response to Tat 10 (Tat 86-102;ESKKEVASKAETDPCDA) with the magnitude of response of 540 SFU/10⁶ PBMCs.

The immunodominant region in Thais is Tat 4 (Tat 30-49; CCWHCQLCFLKKGLGISYGR) and Tat 2(Tat12-29; NHPGSQPTTACSKCYCKK) may be a novel epitope.

The IFN- γ -Elispot responses to individual Tat peptides of 7 patients were shown in figure 9A-9G.

Table 3: IFN- γ -Elispot responses to individual Tat peptides

Initial	Peptide No.	Region	Amino acid sequence of overlapping peptide	The magnitude of responses (SFU/ 10^6 PBMCs)
PA	2	Tat 12-29	NHPGSQPTTACSKCYCKK	204
	3	Tat 20-39	TACSKCYCKKCCWHCQLCFL	824
	4	Tat 30-49	CCWHCQLCFLKKGLGISYGR	592
KK	4	Tat 30-49	CCWHCQLCFLKKGLGISYGR	672
TS	10	Tat 86-102	ESKKEVASKAETDPCDA	540
JL	2	Tat 21-29	NHPGSQPTTACSKCYCKK	308
	3	Tat 20-39	TACSKCYCKKCCWHCQLCFL	324
	4	Tat 30-49	CCWHCQLCFLKKGLGISYGR	176
CY	4	Tat 30-49	CCWHCQLCFLKKGLGISYGR	704
ST	4	Tat 30-49	CCWHCQLCFLKKGLGISYGR	484
	5	Tat 40-56	KKGLGISYGRKKRKHRR	128
RM	1	Tat 1-21	MELVDPNLEPWNHPGSQPTTA	1,264
	3	Tat 20-39	TACSKCYCKKCCWHCQLCFL	240
	4	Tat 30-49	CCWHCQLCFLKKGLGISYGR	436

Figure 8: The number of patients who have IFN- γ -Elispot responses to individual Tat peptides (N=20)

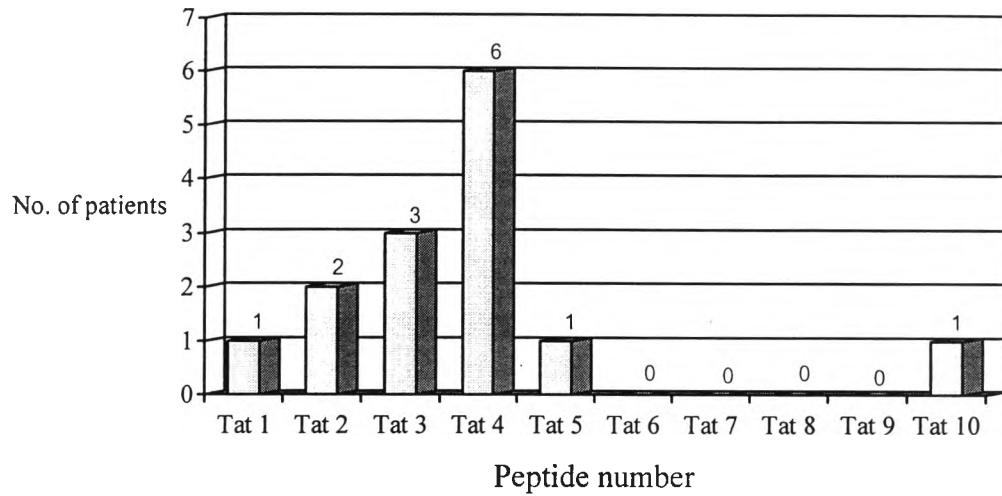
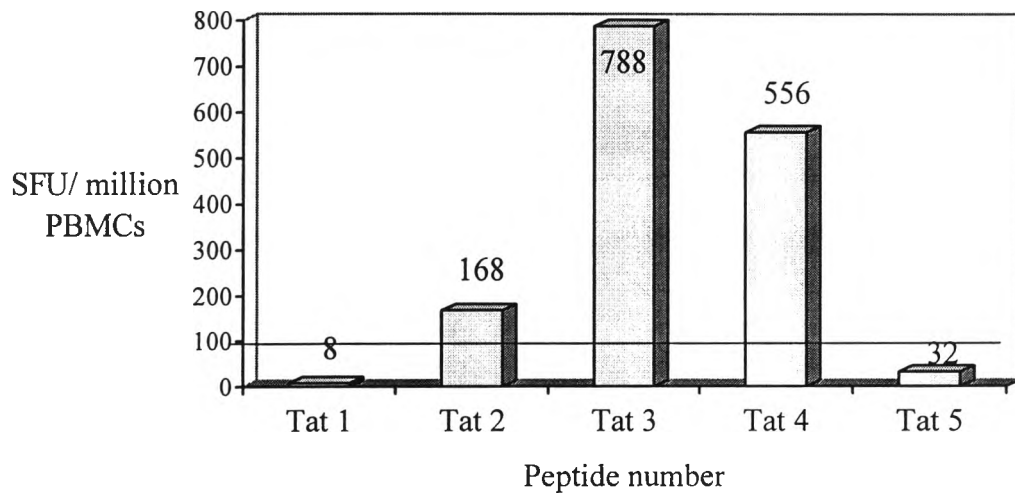
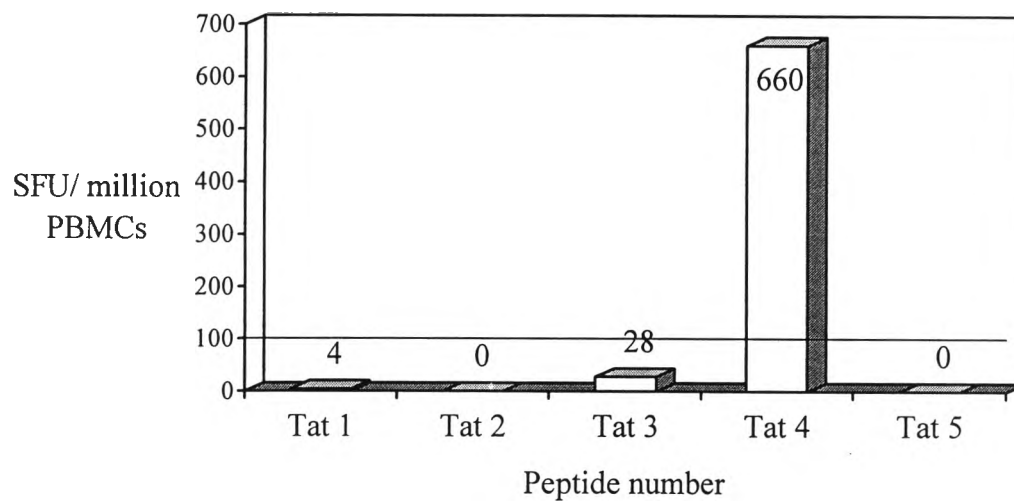


Figure 9: IFN- γ -Elispot responses to individual Tat peptides in each subject (Background subtracted)

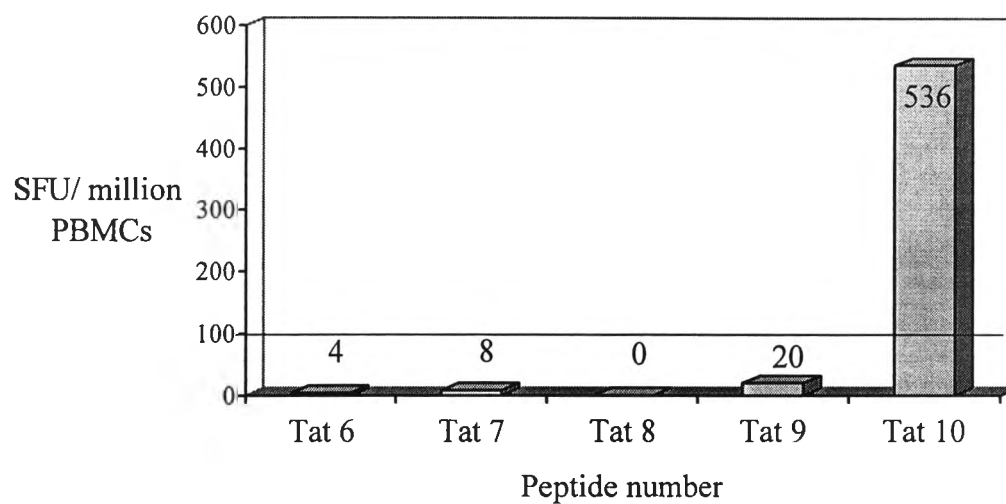
9 A. Subject: PA (CD4+ T cell, 968 cells/ cu.mm.; HIV-RNA, 4,878 copies/mL)

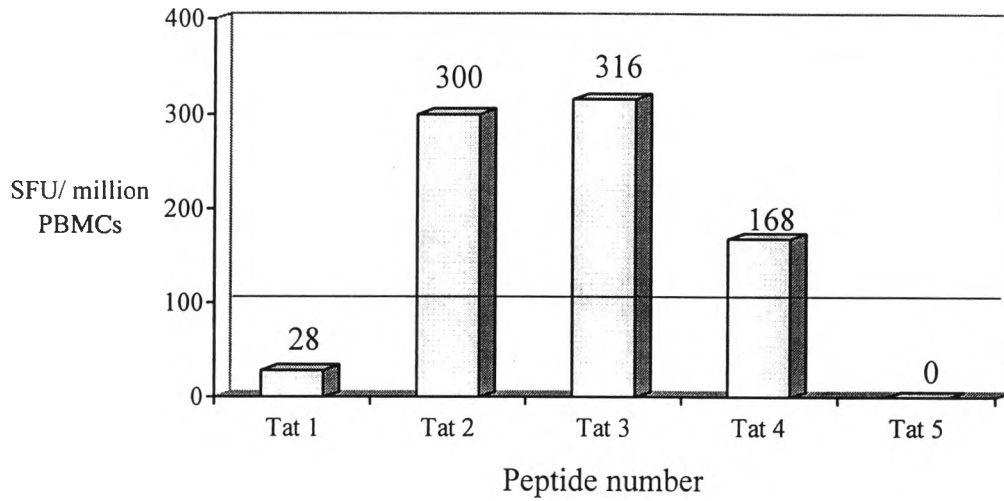
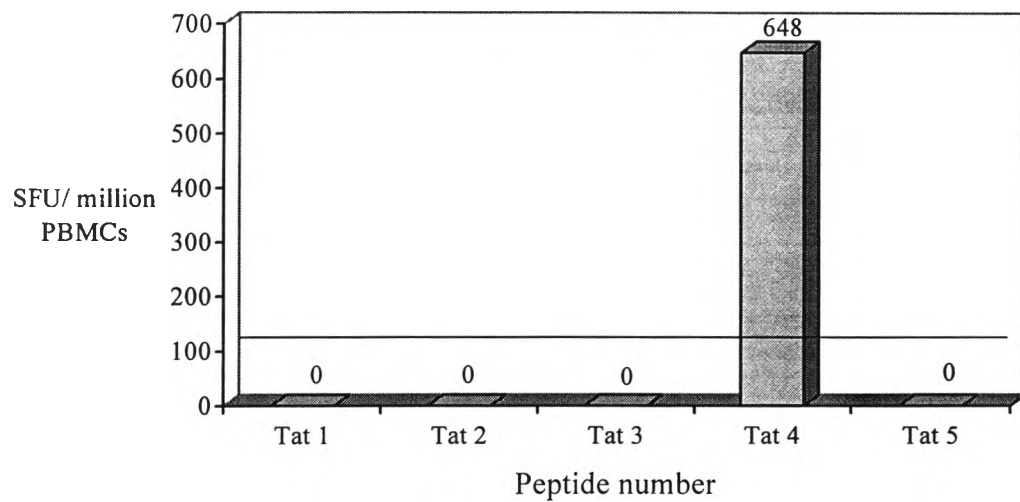


9 B: Subject: KK (CD4+ T cell, 482 cells/ cu.mm.; HIV-RNA, 10,268 copies/mL)



9 C. Subject: TS (CD4+ T cell, 435 cells/ cu.mm.; HIV-RNA, 14,638 copies/mL)



9 D. Subject: JL (CD4+ T cell, 429 cells/ cu.mm.; HIV-RNA, 16,526 copies/mL)**9 E. Subject: CY (CD4+ T cell, 303 cells/ cu.mm.; HIV-RNA, >500,000 copies/mL)**

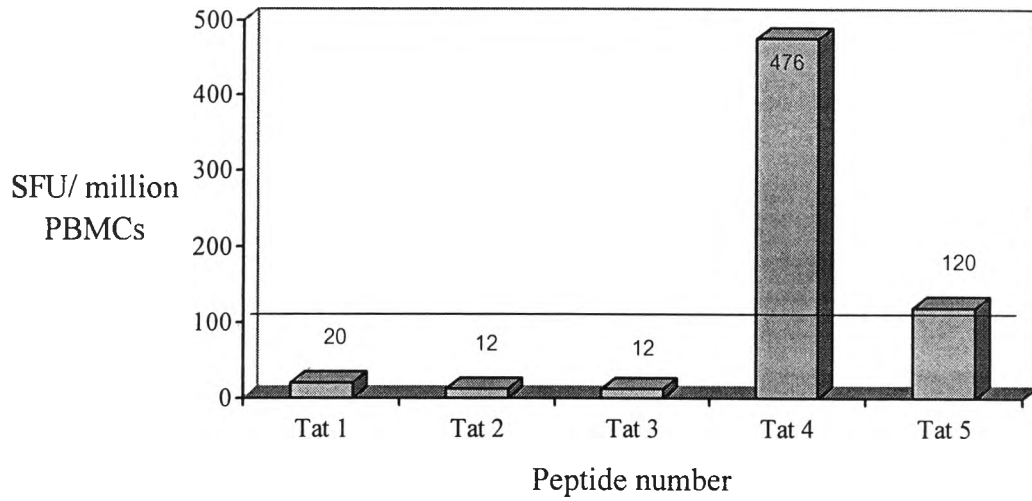
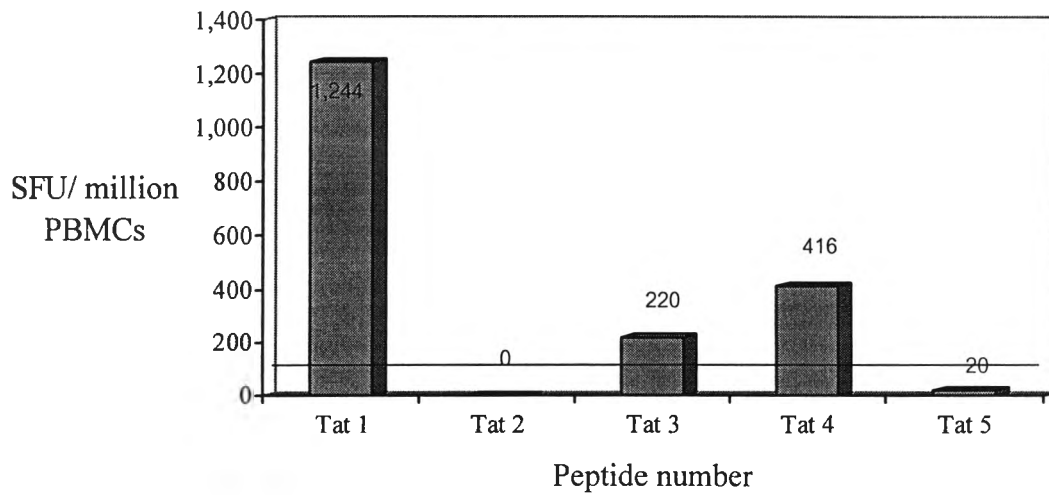
9 F. Subject: ST (CD4+ T cell, 487 cells/ cu.mm.; HIV-RNA, 4,954 copies/mL)**9 G. Subject: RM (CD4+ T cell, 454 cells/ cu.mm.; HIV-RNA, 3,761 copies/mL)**

Figure 10: The number of patients who have IFN- γ -Elispot responses to individual Tat 1, 2, and 3 peptides (N=7)

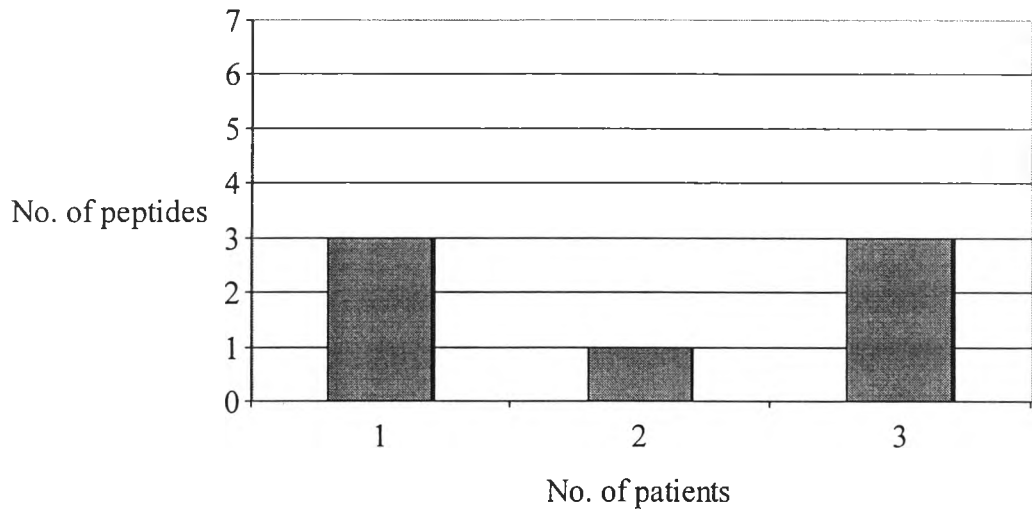


Figure 11: Summary results of CTL epitope mapping based on IFN- γ -Elispot assay in HIV-1 CRF01 AE Tat (“—” represents the CTL epitope which responded by one patient)

