

CHAPTER IV

RESULTS

1. Clinical characteristics

The clinical characteristics were summarized in Table 2. All 25 patients were asymptomatic and antiretroviral treatment naïve. The majority (19/25 or 76%) were heterosexuals and 6/25 (24%) were homosexuals. Based on HIV-1 genotyping and genotyping analysis, 23 naïve infected with subtype A/E, 2 were infected with subtype B. The CD4+ T lymphocytes were ranged from 307 to 978 cells/cu.mm. with mean of 540 cells/cu.mm. Plasma HIV-RNA ranged from 886 to 57,022 copies/ml with the median of 6,936 copies/ml.

2. Cross-clade CTL assay

Of 25 patients, all (100%) showed CTL activity against HIV-1 gag A recombinant vaccinia infected target cells, whereas 16/25 (64%) showed CTL responses against HIV-1 gag B. Among the confirmed subtype A/E infected 23 patients, 19/23 (83%) found CTL against HIV-1 pol A infected targets. Two of two subtype B infected patients had CTL against HIV-1 gag B and but not against HIV-1 pol B (Table 3).

23 subtype A/E infected patients (Table 3), 14 of 23 or 61% showed cross-clade of CTL against HIV-1 gag, CTL activity is (Figure 4). Five of 23 or 17% (Figure 5) had cross-clade HIV-1 pol CTL activity.

Table 2 **Clinical information for naive asymptomatic HIV-1-infected Thai patients**

Patients no.	Initial name of patients	HIV-1 subtype (clade)	Risk factor	CD4+ T cells (cells/cu.mm.)	HIV-RNA (copies/ml)
1	JL	A/E	Heterosexual	451	7502
2	PK	B'	Heterosexual	393	12883
3	TS	A/E	Heterosexual	481	7627
4	DK	A/E	Homosexual	832	5517
5	PW	A/E	Heterosexual	317	18334
6	TW	A/E	Heterosexual	556	886
7	PC	A/E	Heterosexual	546	8185
8	PU	A/E	Heterosexual	660	9456
9	AP	A/E	Heterosexual	541	2986
10	SU	A/E	Homosexual	468	20167
11	CY	A/E	Heterosexual	516	10841
12	KP	A/E	Heterosexual	879	1964
13	OK	B'	Homosexual	307	1341
14	TB	A/E	Heterosexual	607	6936
15	KM	A/E	Heterosexual	371	4905
16	CT	A/E	Homosexual	684	23221
17	AJ	A/E	Heterosexual	487	1141
18	SP	A/E	Homosexual	375	4791
19	JM	A/E	Heterosexual	436	2693
20	KK	A/E	Heterosexual	650	2974
21	ST	A/E	Heterosexual	431	11350
22	AO	A/E	Heterosexual	764	57022
23	PA	A/E	Heterosexual	978	2789
24	TI	A/E	Homosexual	338	28608
25	SN	AE	Heterosexual	439	2733

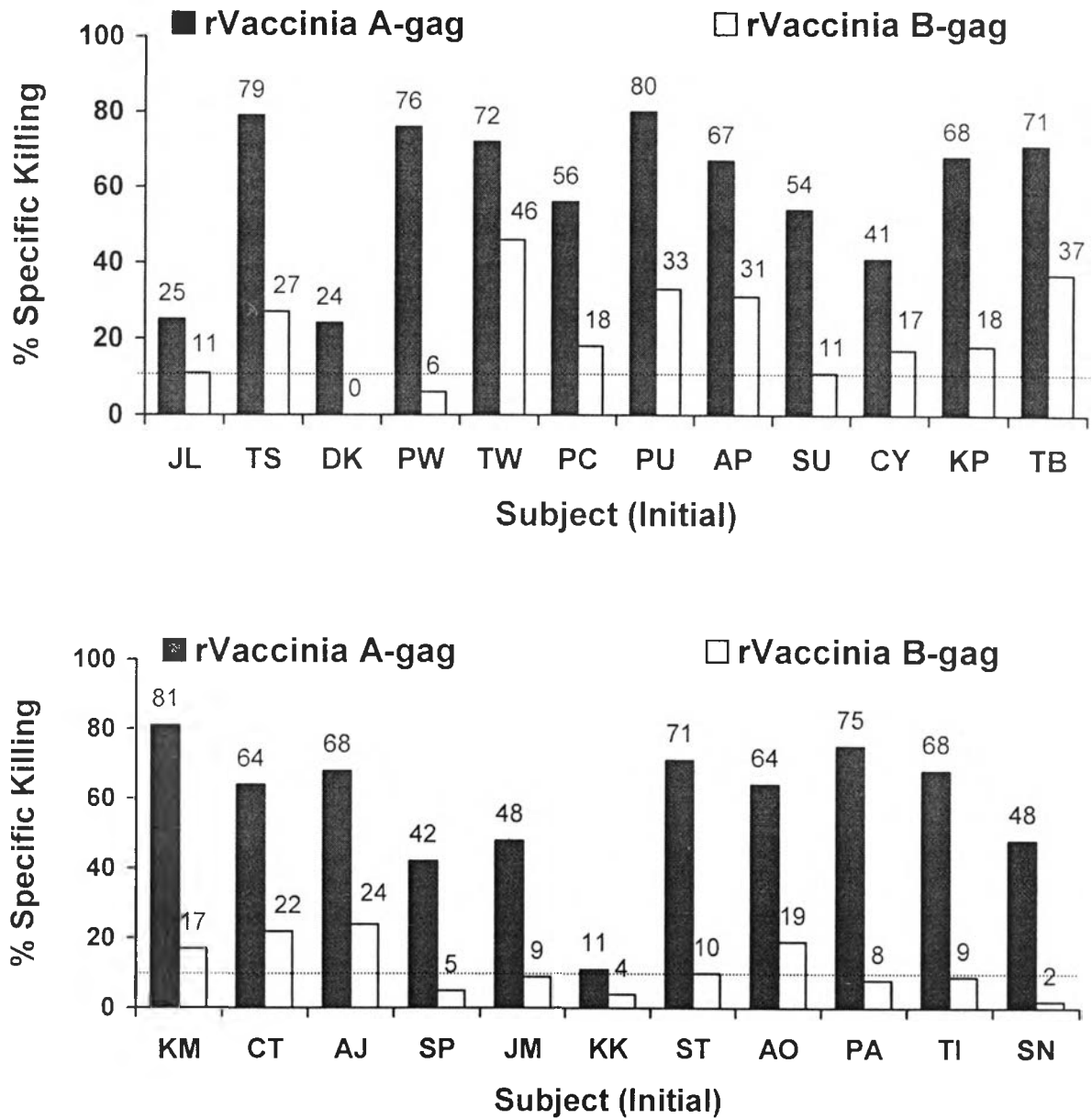
Table 3 Results of cross-clade CTL activity

No.	Initial name of patients	% Specific cytotoxic lysis							
		gag-A		Gag-B		Pol-A		pol-B	
		100:1	50:1	100:1	50:1	100:1	50:1	100:1	50:1
1	JL	14	25	13	11	22	22	8	5
2*	PK	34	27	15	12	29	35	5	7
3	TS	80	79	28	27	54	48	11	11
4	DK	19	24	0	0	13	11	0	0
5	PW	91	76	5	6	72	69	1	2
6	TW	71	72	51	46	68	75	10	13
7	PC	60	56	22	18	65	60	7	5
8	PU	83	80	31	33	74	74	11	13
9	AP	64	67	32	31	64	65	4	3
10	SU	60	54	19	11	35	33	20	23
11	CY	34	41	11	17	24	22	2	3
12	KP	65	68	20	18	61	61	8	9
13*	OK	42	51	41	26	55	58	6	4
14	TB	73	71	40	37	0	0	2	0
15	KM	74	81	20	17	72	72	30	31
16	CT	59	64	21	22	51	52	5	5
17	AJ	75	68	26	24	54	49	8	9
18	SP	42	42	6	5	45	47	1	0
19	JM	48	48	10	9	55	55	2	0
20	KK	17	11	6	4	3	1	2	0
21	ST	70	71	9	10	80	81	0	3
22	AO	64	64	25	19	53	51	13	8
23	PA	77	75	10	8	6	6	0	4
24	TI	62	68	9	11	75	68	3	1
25	SN	58	48	2	2	1	0	1	0

* The patient who infected with HIV-1 subtype B

All data were values subtracted with the WT values and all the spontaneous release results were $\leq 30\%$

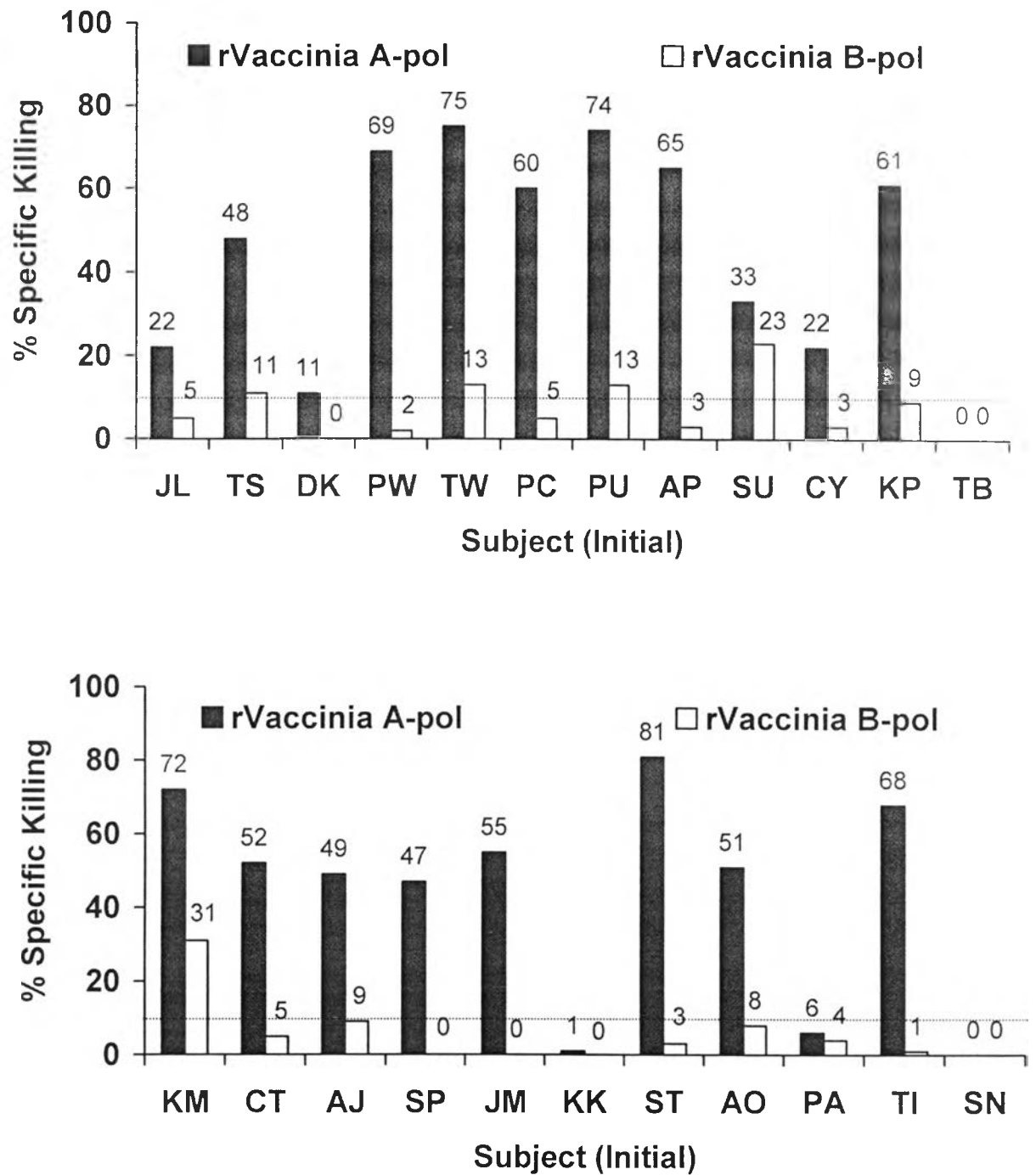
Fig.4 Cross-clade HIV-1 gag CTL activity in HIV-1 clade A/E infection
 N = 14/23 or 61% at E : T 50:1 assay



..... Dashed line represents the positive cut-off of positive specific CTL killing.

All data were values subtracted with the WT values and all the spontaneous release results were $\leq 30\%$

Fig.5 Cross-clade HIV-1 pol CTL activity in HIV-1 clade A/E infection
 N = 5/23 or 22% at E : T 50:1 assay



..... Dashed line represents the positive cut-off of positive specific CTL killing.

All data were values subtracted with the WT values and all the spontaneous release results were ≤ 30%

3. Gag A epitope mapping CTL assay

The results of gag A epitope mapping of each patients were shown in Table 4. Twenty-three HIV-1 subtype A/E infected patients who showed HIV-1 specific CTL killing against recombinant HIV-1 gag A vaccinia infected targets, were mapped to gag A CTL epitopes.

A total of 19 HIV-1 subtype A/E infected patients were able to define HIV-1 gag A epitopes (Figure 6). Eighteen distinct peptides were found 7 epitopes locate in p17, 10 in p24 region and 1 in p6 region. Four patients were unable to identify any dominant CTL epitope despite recognition of proteins expressed from HIV-1 gag-vaccinia recombinant infected autologous cells (DK, PC, AJ, JM). The most common HIV-1 gag A peptide recognized by 7 of 19 patients (37%) is the amino acid residue 271-290 located in p24 region (patients: PU, CY, KM, CT, KK, ST, AO).

Table 4 HIV-1 gag A CTL epitope recognition

No.	Initial name of patients	Gag A peptide	% Specific cytotoxic lysis
1	JL	Gag 3792 (p24) : aa 301-320 FKTLRAEQATQEVKGWMTET	62%
2	PK	ND	
3	TS	Gag 3743 (p24) : aa 201-220 LKDTINEEAAEWDR LHPVHA	14%
4	DK	No clear dominant peptide despite gag recognition	-
5	PW	Gag 3792 (p24) : aa 221-240 GPVAPGQMREPRGSDIAGTT	74%

ND = not done



No.	Initial name of patients	Gag A peptide	% Specific cytotoxic lysis	
6	TW	Gag 3773 (p17) : 11-30 GKLDWEKIRLRPGGKKKYR	19%	
		Gag 3779 (p17) : aa 71-90 GTEELRSLYNTVATLYCVHQ	33%	
		Gag 3788 (p24) : 161-180 EKALSPEVIPMFSALSEGAT	78%	
		Gag 3789 (p24) : 171-190 MFSALSEGATPQDLNMLNI	49%	
		Gag 3765 (p24) : aa 419-438 HQMKDCTERQANFLGKIWPS	53%	
7	PC	No clear dominant peptide despite gag recognition		
8	PU	Gag 3750 (p24) : aa 271-290 NKIVRMYSVPSILDIKQGPK	53%	
9	AP	Gag 3774 (p17) : aa 21-40 LRPGGKKKYRLKHLVWASRE	40%	
		Gag 3786 (p17) : aa 140-160 GQMIHQSLSPRTLNAWVKVIE	62%	
10	SU	Gag 3780 (p17) : aa 81-100 TVATLYCVHQRIEVKDTKEA	21%	
		Gag 3797 (p24) : aa 301-320 FKTLRAEQATQEVKGWMTET	36%	
11	CY	Gag 3772 (p17) : aa 1-20 MGARASVLSGGKLDWEKIR	25%	
		Gag 3774 (p17) : aa 21-40 LRPGGKKKYRLKHLVWASRE	40%	
		Gag 3775 (p17) : aa 31-50 LKHLVWASRELERFALNPSL	41%	
		Gag 3776 (p17) : aa 41-60 LERFALNPSLLETTEGCQOI	37%	

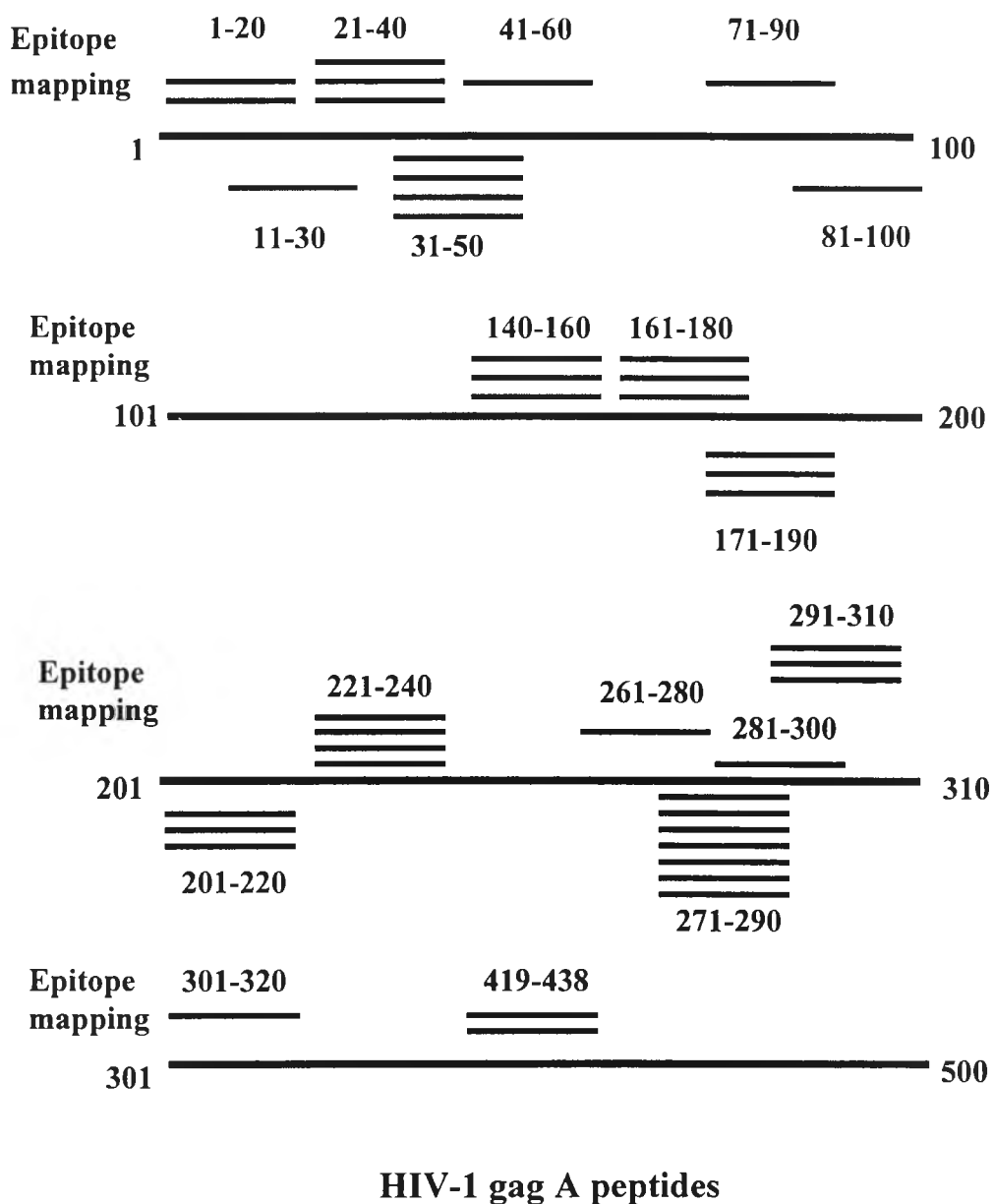
No.	Initial name of patients	Gag A peptide	% Specific cytotoxic lysis
11	CY	Gag 3750 (p24) : aa 271-290 NKIVRMYSPPVSILDIKQGPK	46%
12	KP	Gag 3786 (p24) : aa 140-160 GQMIHQSLSPRTLNAWVKVIE Gag 3792 (P24) : aa 221-240 GPVAPGQMREPRGSDIAGTT	28% 56%
13	OK	ND	
14	TB	Gag 3751 (P24) : aa 281-300 SILDIKQGPKEPFRDYVDRF Gag 3752 (P24) : aa 291-310 EPFRDYVDRFFKTLRAEQAT	56% 41%
15	KM	Gag 3789 (p24) : aa 171-20 MFSALSEGATPQDLNMMLNI Gag 3750 (p24) : 271-290 NKIVRMYSPPVSILDIKQGPK	34% 39%
16	CT	Gag 3788 (p24) : aa 161-180 EKALSPEVIPMFSALSEGAT Gag 3750 (p24) : aa 271-290 NKIVRMYSPPVSILDIKQGPK	33% 43%
17	AJ	No clear dominant peptide despite gag recognition	-
18	SP	Gag 3775 (p17) : aa 31-50 LKHLVWASRELERFALNPSL	57%
19	JM	No clear dominant peptide despite gag recognition	-

ND = not done

No.	Initial name of patients	Gag A peptide	% Specific cytotoxic lysis
20	KK	Gag 3786 (p24) : aa 140-160 GQMIHQSLSPRTLNAWVKVIE	22%
		Gag 3796 (p24) : aa 261-280 IYKRWMILGLNKIVRMYSVPV	73%
		Gag 3750 (p24) : aa 271-290 NKIVRMYSPPVSILDIKQGPK	34%
21	ST	Gag 3750 (p24) : aa 271-290 NKIVRMYSPPVSILDIKQGPK	24%
22	AO	Gag 3788 (p24) : aa 161-180 EKALSPEVIPMFSALSEGAT	32%
		Gag 3789 (p24) : aa 171-190 MFSALSEGATPQDLNMMLNI	16%
		Gag 3750 (p24) : aa 271-290 NKIVRMYSPPVSILDIKQGPK	80%
		Gag 3752 (p24) : aa 291-310 EPFRDYVDRFFKTLRAEQAT	72%
23	PA	Gag 3774 (p17) : aa 21-40 LRPGGKKKYRLKHLVWASRE	73%
		Gag 3743 (p24) : aa 201-220 LKDTINEEAAEWDRHLHPVHA	50%
24	TI	Gag 3775 (P17) : aa 31-50 LKHLVWASRELERFALNPSL	81%
		Gag 3743 (P24) : aa 201-220 LKDTINEEAAEWDRHLHPVHA	92%
		Gag 3752 (P24) : aa 291-310 EPFRDYVDRFFKTLRAEQAT	33%

No.	Initial name of patients	Gag A peptide	% Specific cytotoxic lysis
25	SN	Gag 3772 (p17) : aa 1-20 MGARASVLSGGKLDWEKIR	52%
		Gag 3775 (P17) : aa 31-50 LKHLVWASRELERFALNPSL	44%
		Gag 3792 (p24) : aa 301-320 FKTLRAEQATQEVKGWMTET	32%
		Gag 3765 (p24) : aa 419-438 HQMKDCTERQANFLGKIWPS	46%

Fig. 6 CTL epitope mapping in HIV-1 clade A/E infection (N = 19)
 Eighteen epitopes were identified, 7 epitopes locate in p17 (1-140), 10 in p24 region (141-400) and 1 in p6 region (401-500). The most common epitope is the amino acid 271-290 (p24) found in 7 of 19 patients (37%).

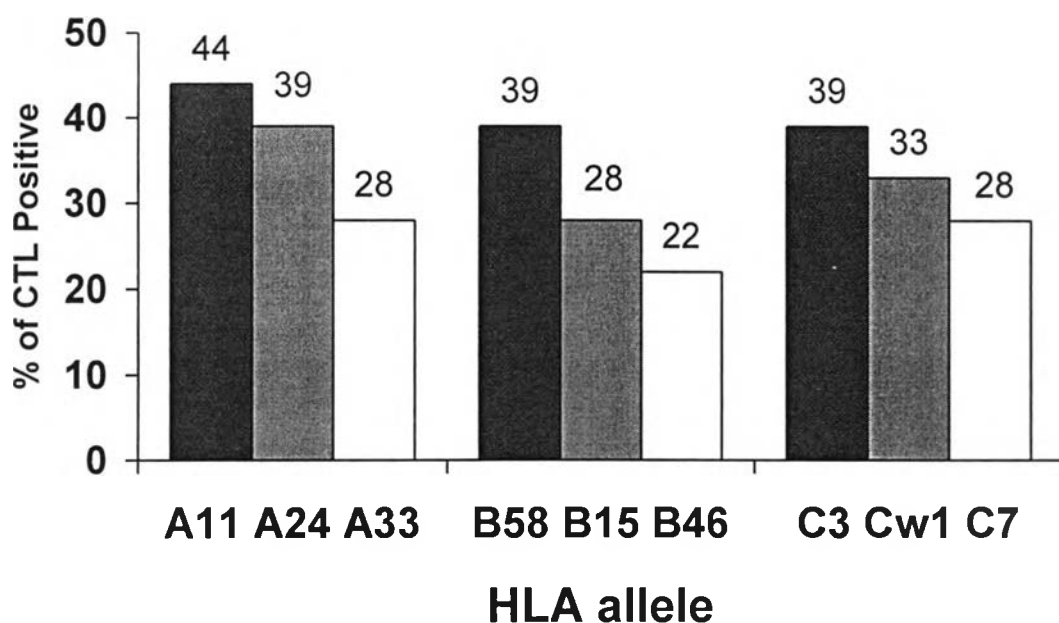


———— The line represents 1 CTL epitope from each patient

4. HLA class I genotypic

In this study, we tested the HLA class I genotyping in eighteen patients. The preliminary data showed in Figure 7. We found the majority of the HLA-A11 about 8 of 18 or 44%, HLA-A24 are 7 of 18 or 39% and HLA-A33 is 5 of 18 or 28%. In HLA-B showed 7 of 18 or 39% is the HLA-B58, HLA-B15 showed 5 of 18 or 28% whereas, HLA-B46 showed 4 of 18 or 22%. In HLA-C data, we found HLA-C3 is 7 of 18 or 39%, HLA-Cw1 is 33% and HLA-C7 is 5 of 18 or 28%.

Fig. 7 The three most common HLA class I molecule found in 18 patients.
HLA A = A11, A24, A33; HLA B = B58, B15, B46; HLA C = C3, Cw1, C7



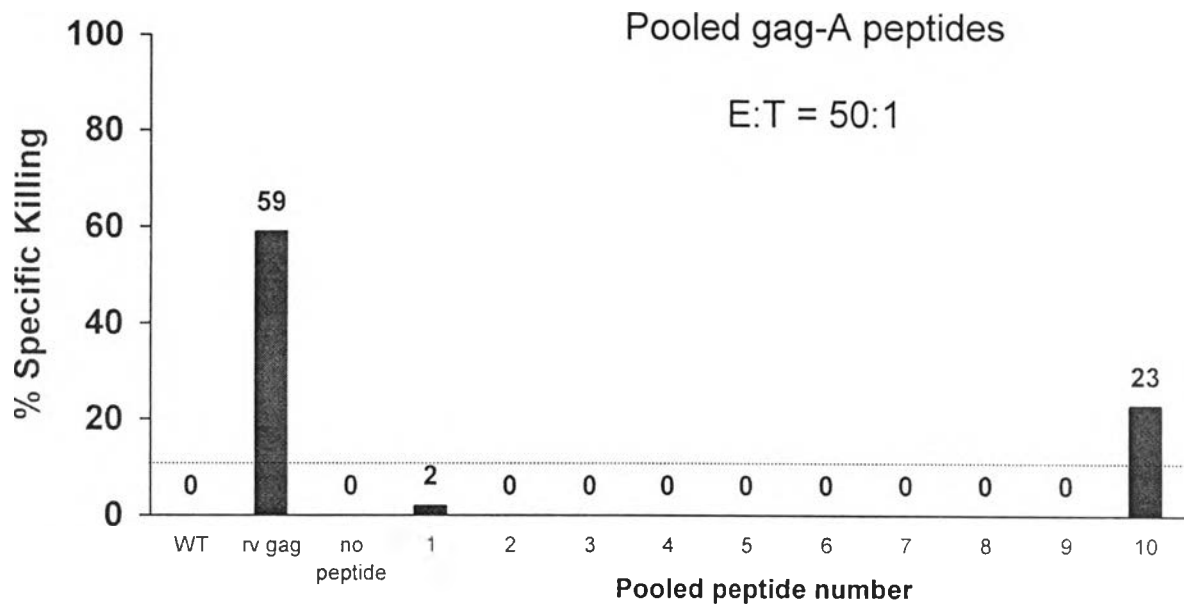
5. HLA-restriction

In addition to the proposed thesis, three patients: PU, AP and KM were further studied non-randomizedly to determine HLA-restriction of those who recognized CTL epitopes using HLA-matched and mis-matched BLCLs as target cells. The results in details of each individual included HIV-1 gag A CTL epitope mappings and HLA class I restriction were summarized as followings and shown in Figure 8, 9, 10, 11, 12, 13, 14 and Table 5, 6, 7, 8 .

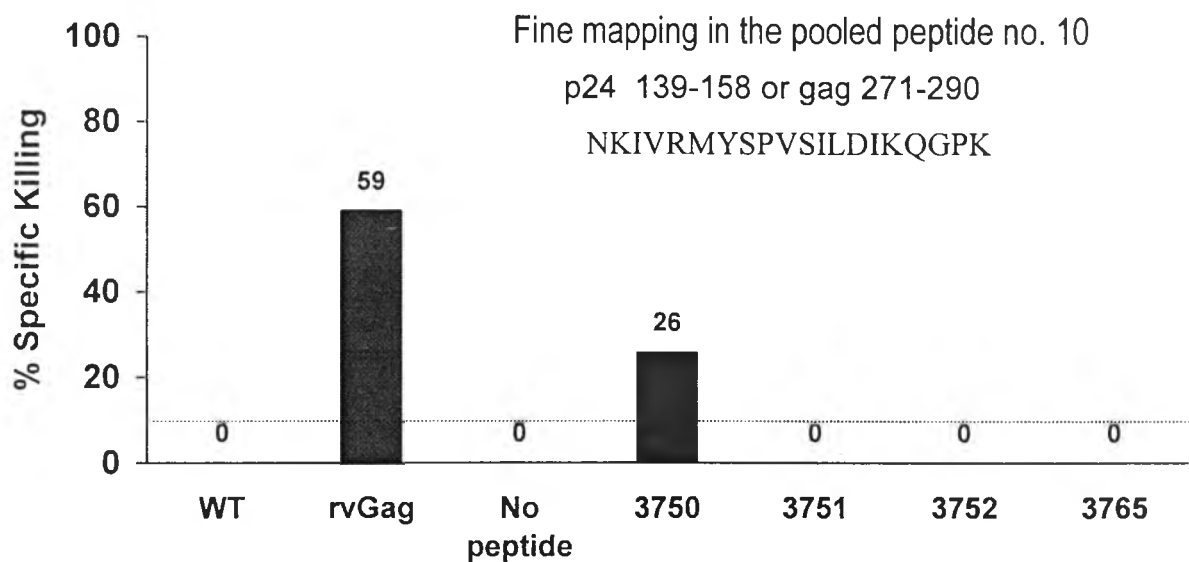
Patient PU As shown in Figure 8a, among 10 pooled HIV-1 gag A overlapping peptides, no. 10 showed a significant and the highest specific CTL killing. When individual peptides: no. 3750, 3751, 3752, and 3765, were tested, only HIV-1 gag A peptide no. 3750 has shown a similar magnitude of specific killing (Figure 8b). This indicates that patient PU recognized this CTL epitope which located in p24 region at residues gag 271-290: **NKIVRMYSPPVSILIKQGP**K. The HLA class I restriction results were shown in the Table 5 and Figure 9. He carries HLA A11, A3303; B55, B40; Cw1, C3. Only Cw1 matched heterologous BLCLs containing experiments revealed a high and comparable level of specific CTL killing as of the autologous BLCL results. This concludes that p24 gag 271-290 epitope (or p24 residues 139-158) is presented and restricted by HLA Cw1 in this patient.

Fig.8 HIV-1 gag peptide epitope mapping results of patient PU with CD4+ = 660 cells/cu.mm. and HIV-1 RNA = 7903 copies/ml

(8a) Specific CTL for 10 pooled HIV-1 gag A overlapping peptides, no. 10 showed a significant and the highest killing at E : T 50:1 assay



(8b) Specific CTL of individual peptide, no. 3750 showed a significant specific killing at E : T 50:1 assay



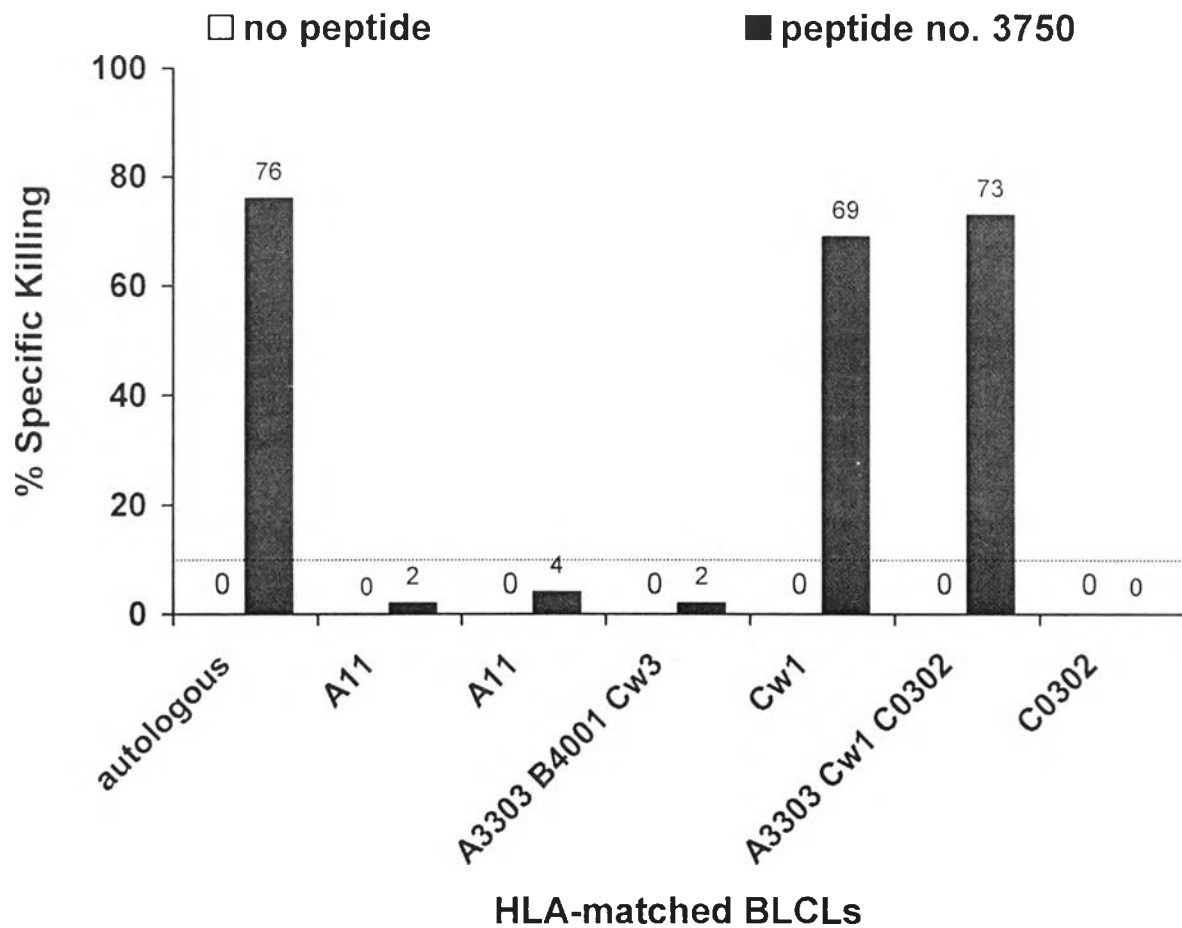
..... Dashed line represents the positive cut-off of positive specific CTL killing.
All data were values subtracted with the WT values and all the spontaneous release results were $\leq 30\%$

Table 5 HLA-restriction results of patient PU on peptide no. 3750 (p24, aa 271-290 : NKIVRMYSVPSILDIKQGPK) HLA = A11,A3303 ; B55,B40 ; Cw1,C3

Initial of BLCL	HLA typing	HLA-matched BLCLs	% Specific cytotoxic lysis
Autologous	A11,A3303 B55,B40 Cw1,C3	A11,A3303 B55,B40 Cw1,C3	76 %
No. 1	A2, <u>A11</u> B7,B6	A11	2 %
No. 2	<u>A11</u> ,A29 B18,B35	A11	4 %
No. 3	A24, <u>A3303</u> <u>B4001</u> ,B44032 Cw*07016/6, <u>Cw3</u>	A3303 B4001 Cw3	2 %
No. 4	A0201,A24 B4601.B15 Cw12, <u>Cw1</u>	Cw1	69 %
No. 5	A0201, <u>A3303</u> B46,B5801 <u>Cw1.C0302</u>	A3303 Cw1, C0302	73 %
No. 6	A24,- B5801,B1502 C8, <u>C0302</u>	C0302	0 %

Interpretation: Peptide gag A (271-290) was presented and restricted by HLA Cw1

Fig.9 HLA-restriction of patient PU with peptide no. 3750 (p24, aa 271-290 : NKIVRMYSPPVSILDIKQGPK) at E : T 30:1 assay. HLA = A11,A3303 ; B55,B40 ; Cw1,C3



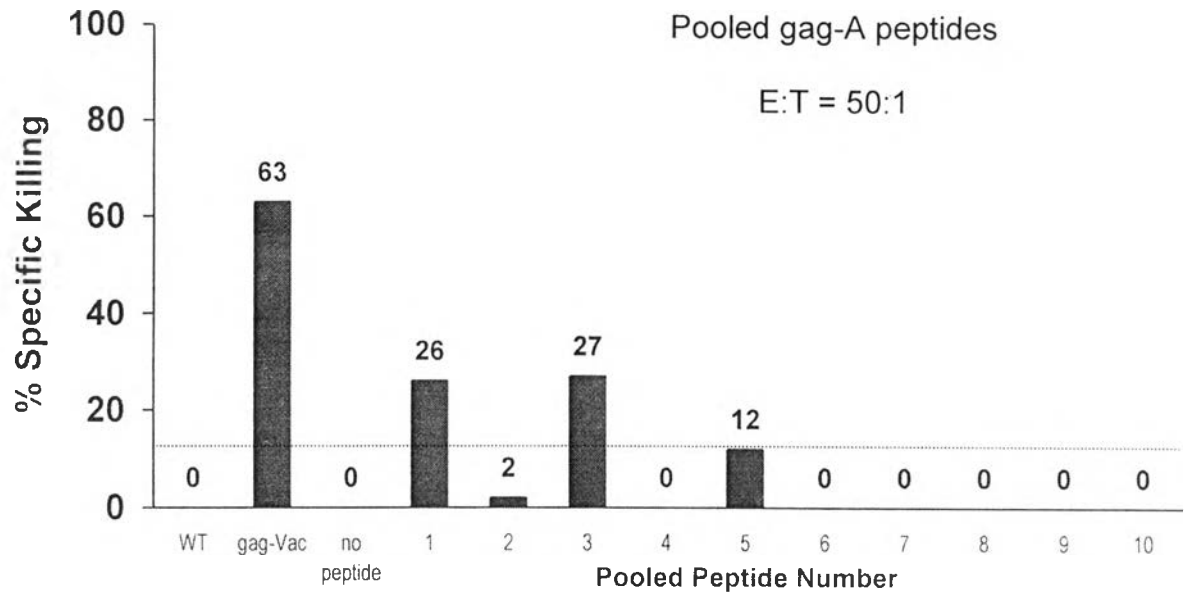
Interpretation: Peptide gag A no.3750 (271-290) was presented and restricted by HLA Cw1

All data were values subtracted with the WT values and all the spontaneous release results were $\leq 30\%$

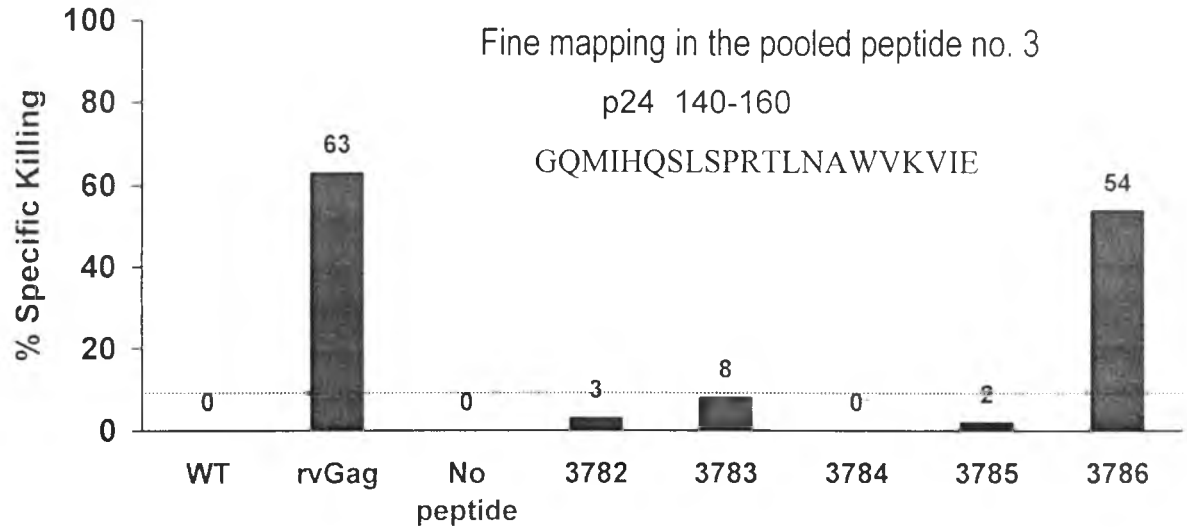
Patient AP As shown in Figure 10a, this patient recognized pooled gag A peptides no. 1 and 3 were showed of CTL specific killing. In additional studies to the proposed thesis, further mapping of peptides pool no.3 (peptide no. 3782, 3783, 3784, 3785 and 3786) was performed. The results showed that peptide no. 3786 was specifically recognized with the specific killing of 54 % (Figure 10b). This peptide is located in p17 region at residues gag 140-160: **GQMIHQSLSPRTLNA WVKVIE**. The HLA class I genotyping results indicated that she carries A1, A2; B5701, B27; Cw6, Cw0702/10. In the matched and mis-matched HLA restriction experiments, peptide gag 140-160 was presented and restricted by HLA A2. (Table 6 and Figure 11).

Fig.10 HIV-1 gag peptide epitope mapping of patient AP with CD4+ = 541 cells/cu.mm. and HIV-1 RNA = 2986 copies/ml.

(10a) Specific CTL for 10 pooled HIV-1 gag A overlapping peptides, no. 1 and no. 3 showed a significant specific killing at E : T 50:1 assay



(10b) Specific CTL of individual peptides, no. 3786 showed a significant specific killing at E : T 50:1 assay



..... Dashed line represents the positive cut-off of positive specific CTL killing.

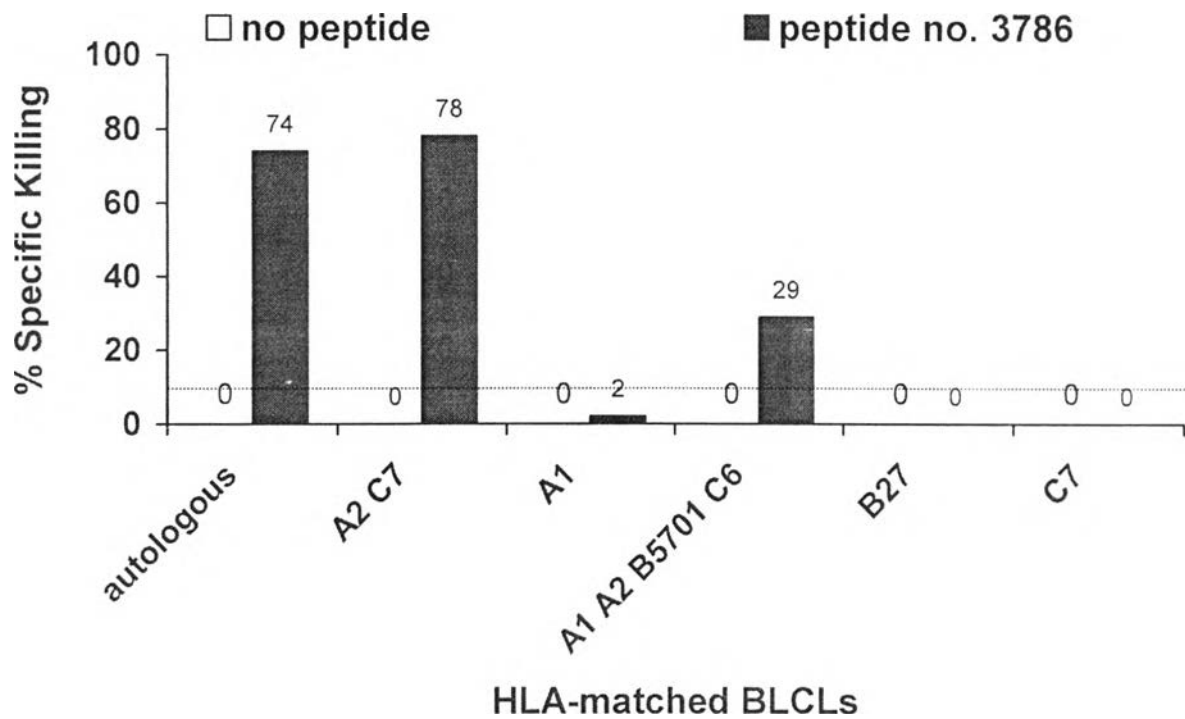
All data were values subtracted with the WT values and all the spontaneous release results were $\leq 30\%$

Table 6 HLA-restriction of patient AP with peptide no. 3786 (p17, aa 140-160 : GQMIHQSLSPRTLNAWVKVIE) HLA = A1,A2 ; B5701,B27 ; Cw6,Cw0702/10

Initial of BLCL	HLA typing	HLA-matched BLCLs	% Specific cytotoxic lysis
Autologous	A1,A2 B5701,B27 Cw6,Cw0702/10	A1,A2 B5701,B27 Cw6,Cw0702/10	74 %
No. 1	A2,A11 B18,B52001 C4,C7	A2 C7	78 %
No. 2	A1.A3 B7,B44	A1	2 %
No. 3	A1,A2 B5701,B1513 C6,C8	A1, A2 B5701 C6	29 %
No. 4	A3.A3303 B27,B40 Cw1,C2	B27	0 %
No. 5	A11,- B40,Bw6 C3,C7	C7	0 %

Interpretation: Peptide gag A (271-290) was presented and restricted by HLA A2

Fig.11 HLA-restriction of patient AP with peptide no. 3786 (p17, aa 140-160 : GQMIHQSLSPRTLNAWVKVIE at E : T 30:1 assay. HLA = A1,A2 ; B5701,B27 ; Cw6,Cw0702/10



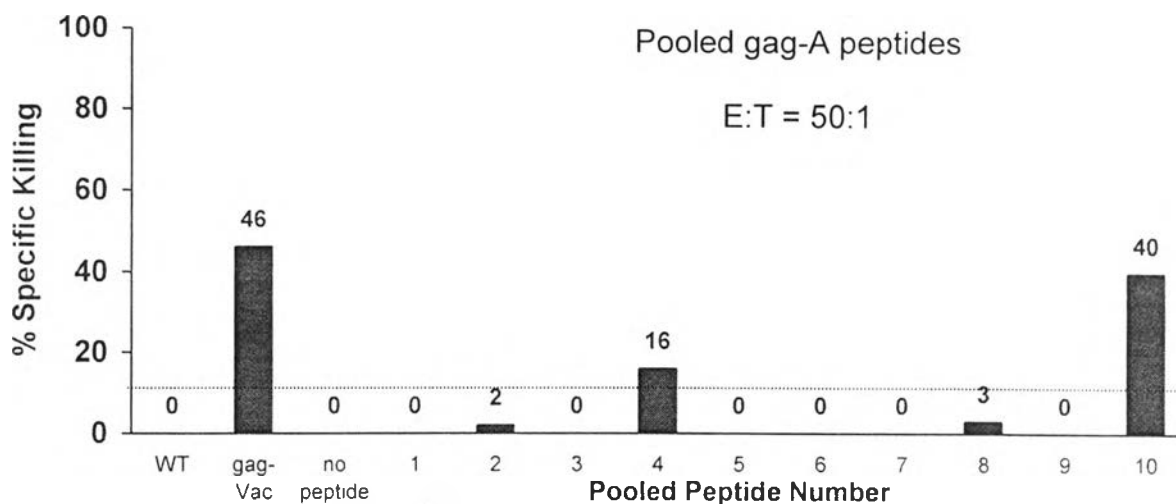
Interpretation: Peptide gag A no.3786 (140-160) was presented and restricted by HLA A2

All data were values subtracted with the WT values and all the spontaneous release results were $\leq 30\%$

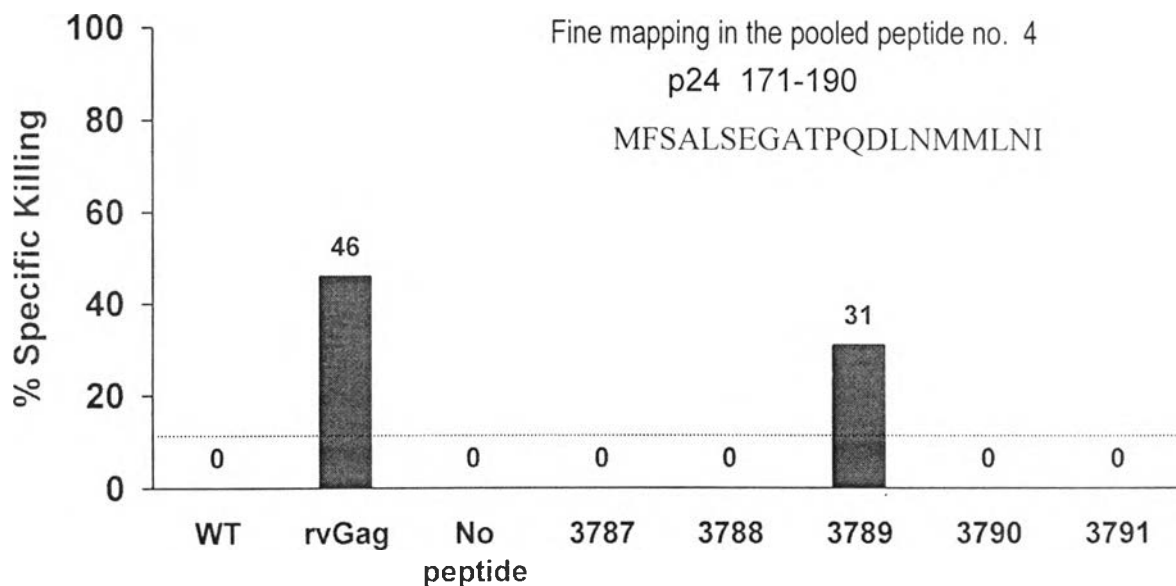
Patient KM This patient recognized pool no. 4 and no. 10 of overlapping truncated HIV-1 gag A peptides with killing of 16% and 40%, respectively (Figure 12a). As shown in Figure 12b and 12c, peptide 3789 of pool no. 4 (HIV-1 gag residue 171-190 located in p24: **MFSALSEGATPQDLNMLNI**) and 3750 of pool no. 10 (HIV-1 gag residue 271-290 located in p24: **NKIVRMYSPPVSLDIKQGP**) pulsed target cells showed comparable specific CTL activities to those of recombinant HIV-1 gag vaccinia infected targets. She carries HLA A0201, A24; B7, B27; Cw1, Cw7. The HLA restriction experiments indicate that the first epitope (gag 171-190) is restricted by HLA B7 (Table 7 and Figure 13), whereas the second (peptide gag 271-290) is restricted by HLA Cw1. (Table 8 and Figure 14).

Fig.12 HIV-1 gag peptide epitope mapping results of patient with CD4+ = 371 cells/cu.mm. and HIV-1 RNA = 4905 copies/ml

(12a) Specific CTL for 10 pooled HIV-1 gag A overlapping peptides, no. 4 and no. 10 showed a significant and the highest killing at E : T 50:1 assay

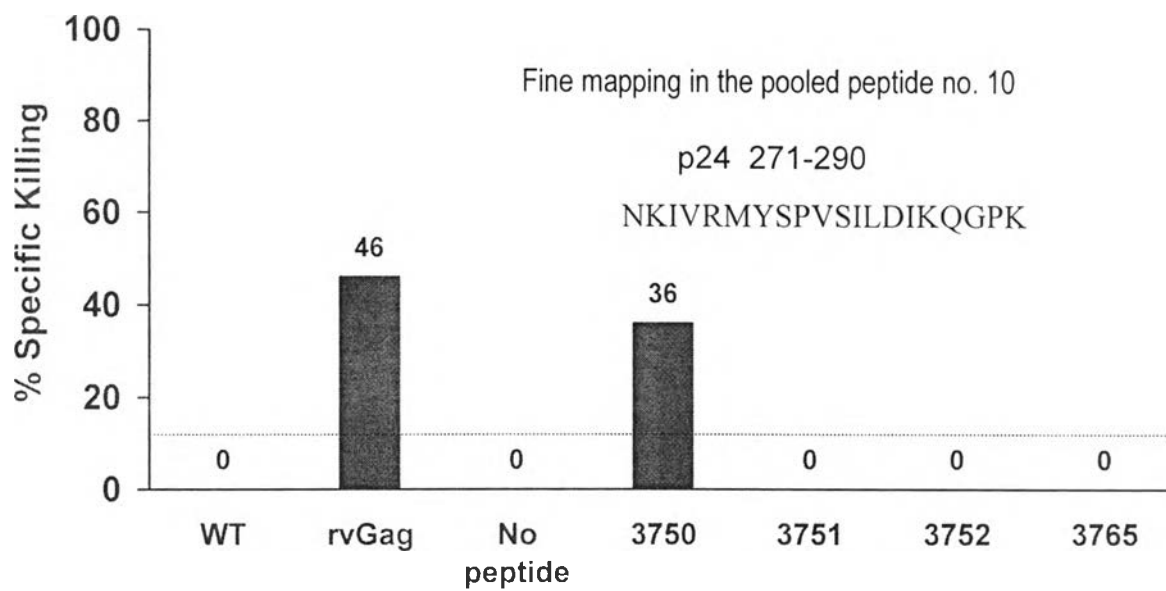


(12b) Specific CTL of individual peptides, no. 3789 showed a significant specific killing at E : T 50:1 assay



..... Dashed line represents the positive cut-off of positive specific CTL killing .
All data were values subtracted with the WT values and all the spontaneous release results were $\leq 30\%$

(12c) Specific CTL of individual peptides, no. 3750 showed a significant specific killing at E : T 50:1 assay



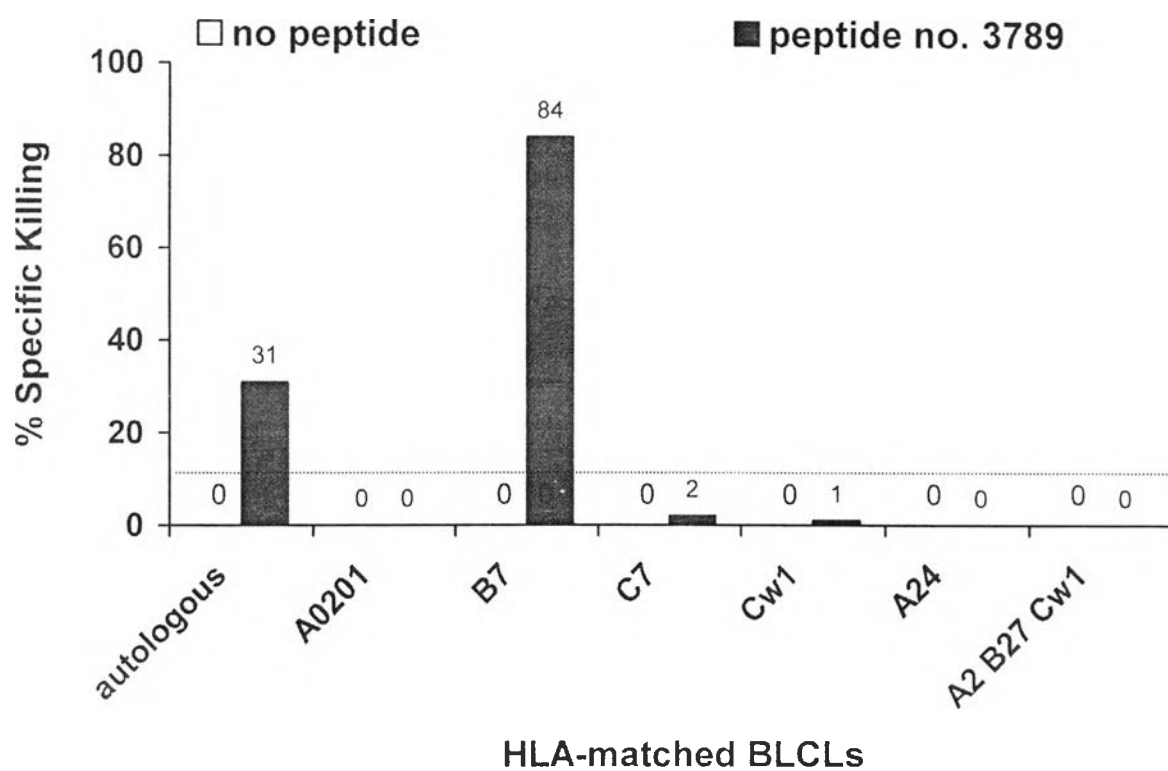
..... Dashed line represents the positive cut-off of positive specific CTL killing.
All data were values subtracted with the WT values and all the spontaneous release results were $\leq 30\%$

Table 7 HLA-restriction of patient KM with peptide no. 3789 (p24, aa 171-200 MFSALSEGATPQDLNMMLNI) HLA = A0201,A24 ; B7,B27 ; Cw1,Cw7

Initial of BLCL	HLA typing	HLA-matched BLCLs	% Specific cytotoxic lysis
Autologous	A0201,A24 B7,B27 Cw1,Cw7	A0201,A24 B7.B27 Cw1,Cw7	31 %
No. 1	A1, A0201 B5701,B1513 C6,C8	A0201	0 %
No. 2	A1,A3 B7 ,B44	B7	84 %
No. 3	A0203,A11 B18,B52001 C4, C7	C7	2 %
No. 4	A11,A3303 B55,B40 Cw1 ,C3	Cw1	1 %
No. 5	A24 .A3303 B18,B5801 C0302,C12	A24	0 %
No. 6	A2 , - B27 ,B46 Cw1	A2, B27 Cw1	0 %

Interpretation: Peptide gag A (140-160) was presented and restricted by HLA B7

Fig.13 HLA-restriction of patient KM with peptide no. 3789 (p24, aa 171-200 MFSALSEGATPQDLNMMLNI) at E : T 30:1 assay. HLA = A0201,A24 ; B7,B27 ; Cw1,Cw7



Interpretation: Peptide gag A no.3789 (171-200) was presented and restricted by HLA B7

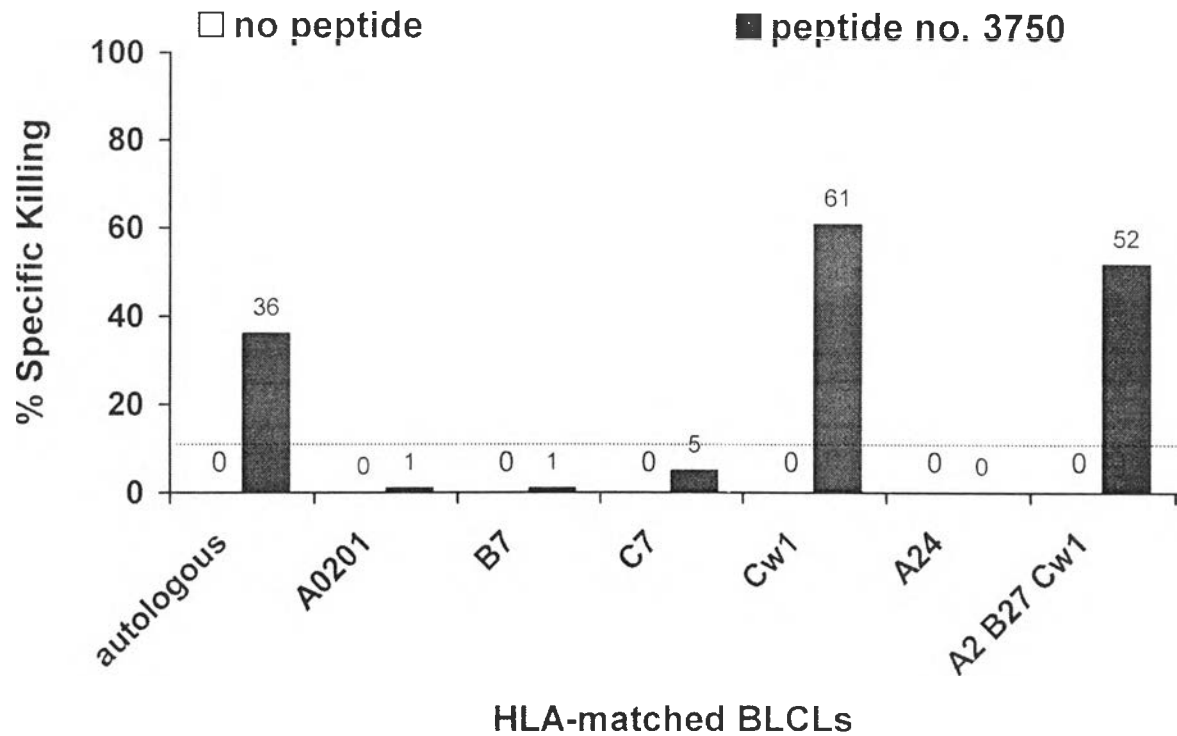
All data were values subtracted with the WT values and all the spontaneous release results were $\leq 30\%$

Table 8 HLA-restriction of patient KM on peptide no. 3750 (p24, 271-290 NKIVRMYPVSI~~L~~DIKQGPK). HLA = A0201,A24 ; B7,B27 ; Cw1,Cw7

Initial of BLCL	HLA typing	HLA-matched BLCLs	% Specific cytotoxic lysis
Autologous	A0201,A24 B7,B27 Cw1,Cw7	A0201,A24 B7,B27 Cw1,Cw7	36 %
No. 1	A1, A0201 B5701,B1513 C6,C8	A0201	1 %
No. 2	A1,A3 B7 ,B44	B7	1 %
No. 3	A0203,A11 B18,B52001 C4, C7	C7	5 %
No. 4	A11,A3303 B55,B40 Cw1 ,C3	Cw1	61 %
No. 5	A24 .A3303 B18,B5801 C0302,C12	A24	0 %
No. 6	A2 ,- B27 ,B46 Cw1	A2 B27 Cw1	52 %

Interpretation: Peptide gag A (171-200) was presented and restricted by HLA Cw1

Fig. 14 HLA-restriction of patient KM with peptide no. 3750 (p24, 271-290 NKIVRMYSILDIKQGPK) at E : T 30:1 assay. HLA = A0201,A24 ; B7,B27 ; Cw1,Cw7



Interpretation: Peptide gag A no. 3750 (271-290) was presented and restricted by HLA Cw1

All data were values subtracted with the WT values and all the spontaneous release results were $\leq 30\%$

6. Example for raw data of patient PU

In this study, we tested the CTL activity by using classical chromium release assay. We were plated effector and target cell in the 96 well, U-bottomed microtiter plate, 200 μ l final volume of each 100 μ l of effector cells concentration in triplicate and 100 μ l of target cells. The ratio of effector to target cell at 100:1 and 50:1. For the total release (TR) or maximum release 5% triton-X 100 (TX 100) was added to the target cells. The chromium-51 release from the wells with culture media R10 only added is used to calculate the spontaneous release (SR) or minimum release. The example for raw data of HLA-restriction of patient PU with peptide no. 3750 showed in Table 9. All the raw data is in the value of mean \pm SD.

Table 9 **Data of HLA restriction of patient PU with peptide no. 3750 (in triplicate)**

Test	Count per Minute (cpm)	Mean \pm SD	% Specific cytotoxic lysis
No peptide, SR, Autologous BLCL	436 427 410	425 \pm 13	-
No peptide, TR, Autologous BLCL	5285 5080 5178	5181 \pm 103	-
No peptide, E:T = 30:1, Autologous BLCL	663 660 655	659 \pm 4	0
No peptide, E:T = 15:1, Autologous, BLCL	609 613 581	601 \pm 17	0
Peptide no. 3750, SR, Autologous BLCL	486 440 471	466 \pm 23	-

Autologous BLCL = HLA A11,A3303 ; B55,B40 ; Cw1,C3



Test	Count per Minute (cpm)	Mean \pm SD	% Specific cytotoxic lysis
Peptide no. 3750, TR, Autologous BLCL	5709 6163 5591	5821 \pm 302	-
Peptide no. 3750, E:T = 30:1, Autologous BLCL	4582 4945 4932	4820 \pm 206	76
Peptide no. 3750, E:T = 15:1, Autologous BLCL	4486 3563 4822	4290 \pm 652	66
No peptide, SR, BLCL 1	346 336 316	333 \pm 15	-
No peptide, TR, BLCL 1	3344 3353 3284	3327 \pm 38	-
No peptide, E:T = 30:1, BLCL 1	482 485 498	488 \pm 9	0
No peptide, E:T = 15:1, BLCL 1	472 475 496	481 \pm 13	0
Peptide no. 3750, SR, BLCL 1	182 191 206	193 \pm 12	-

Autologous BLCL = HLA A11,A3303 ; B55,B40 ; Cw1,C3
BLCL 1 = HLA A2,A11 ; B7,B6

Test	Count per Minute (cpm)	Mean \pm SD	% Specific cytotoxic lysis
Peptide no. 3750, TR, BLCL 1	1072 1063 987	1041 \pm 47	-
Peptide no. 3750, E:T = 30:1, BLCL 1	273 231 243	249 \pm 22	2
Peptide no. 3750, E:T = 15:1, BLCL 1	241 244 221	236 \pm 13	0
No peptide, SR, BLCL 2	393 415 410	406 \pm 12	-
No peptide, TR, BLCL 2	5244 5298 5314	5285 \pm 37	-
No peptide, E:T = 30:1, BLCL 2	589 588 601	593 \pm 7	0
No peptide, E:T = 15:1, BLCL 2	519 474 482	491 \pm 24	0
Peptide no. 3750, SR, BLCL 2	228 230 250	236 \pm 12	-

BLCL 1 = HLA A2,A11 ; B7,B6

BLCL 2 = HLA A11,A29 ; B18,B35

Test	Count per Minute (cpm)	Mean \pm SD	% Specific cytotoxic lysis
Peptide no. 3750, TR, BLCL 2	2137 2123 2196	2152 \pm 39	-
Peptide no. 3750, E:T = 30:1, BLCL 2	398 377 394	389 \pm 11	4
peptide no. 3750 , E:T = 15:1, BLCL 2	340 351 328	340 \pm 12	1
No peptide, SR, BLCL 3	427 469 441	446 \pm 21	-
No peptide, TR, BLCL 3	2829 2827 2761	2806 \pm 39	-
No peptide, E:T = 30:1, BLCL 3	595 585 597	593 \pm 6	0
No peptide, E:T = 15:1, BLCL 3	543 533 587	555 \pm 29	0
Peptide no. 3750, SR, BLCL 3	239 265 256	254 \pm 13	-

BLCL 2 = HLA A11,A29 ; B18,B35

BLCL 3 = HLA A24,A3303 ; B4001,B40032 ; Cw*07016/6,Cw3

Test	Count per Minute (cpm)	Mean \pm SD	% Specific cytotoxic lysis
Peptide no. 3750, TR, BLCL 3	1161 1260 1100	1174 \pm 81	-
Peptide no. 3750, E:T = 30:1, BLCL 3	370 338 385	365 \pm 24	2
peptide no. 3750, E:T = 15:1, BLCL 3	325 279 315	307 \pm 24	0
No peptide, SR, BLCL 4	580 510 538	543 \pm 35	-
No peptide, TR, BLCL 4	5180 5204 5110	5164 \pm 49	-
No peptide, E:T = 30:1, BLCL 4	920 805 881	868 \pm 58	0
No peptide, E:T = 15:1, BLCL 4	711 773 750	731 \pm 31	0
Peptide no. 3750, SR, BLCL 4	504 442 482	476 \pm 31	-

BLCL 3 = HLA A24,A3303 ; B4001.B40032 ; Cw*07016/6,Cw3
BLCL 4 = HLA A0201,A24 ; B4601.B15 ; Cw12,Cw1

Test	Count per Minute (cpm)	Mean \pm SD	% Specific cytotoxic lysis
Peptide no. 3750, TR, BLCL 4	4675 5197 4568	4813 \pm 337	-
Peptide no. 3750. E:T = 30:1, BLCL 4	3487 3934 3916	3779 \pm 253	69
Peptide no. 3750, E:T = 15:1, BLCL 4	3922 3877 3804	3868 \pm 60	71
No peptide, SR. BLCL 5	611 538 529	559 \pm 45	-
No peptide, TR, BLCL 5	4798 4739 4664	4734 \pm 67	-
No peptide, E:T = 30:1, BLCL 5	868 829 881	859 \pm 27	0
No peptide, E:T = 15:1, BLCL 5	786 755 767	770 \pm 16	0
Peptide no. 3750. SR, BLCL 5	443 446 465	452 \pm 12	-

BLCL 4 = HLA A0201,A24 ; B4601,B15 ; Cw12,Cw1

BLCL 5 = HLA A0201,A3303 ; B46,B5801 ; Cw1,C0302

Test	Count per Minute (cpm)	Mean \pm SD	% Specific cytotoxic lysis
Peptide no. 3750, TR, BLCL 5	3965 3914 3954	3944 \pm 27	-
Peptide no. 3750, E:T = 30:1, BLCL 5	3158 3211 3331	3233 \pm 87	73
Peptide no. 3750, E:T = 15:1, BLCL 5	3368 3352 3321	3347 \pm 24	76
No peptide, SR, BLCL 6	273 285 285	281 \pm 7	-
No peptide, TR, BLCL 6	1842 1793 1742	1792 \pm 50	-
No peptide, E:T = 30:1, BLCL 6	365 370 368	368 \pm 3	0
No peptide, E:T = 15:1, BLCL 6	368 341 345	352 \pm 15	0
Peptide no. 3750, SR, BLCL 6	286 290 311	296 \pm 13	-

BLCL 5 = HLA A0201,A3303 ; B46,B5801 ; Cw1,C0302

BLCL 6 = HLA A24,- ; B5801,B1502 ; C8,C0302

Test	Count per Minute (cpm)	Mean \pm SD	% Specific cytotoxic lysis
Peptide no. 3750, TR, BLCL 6	1907 1926 1957	1930 \pm 25	-
Peptide no. 3750, E:T = 30:1, BLCL 6	389 358 378	375 \pm 16	0
Peptide no. 3750, E:T = 15:1, BLCL 6	354 381 356	364 \pm 15	0

BLCL 6 = HLA A24,- ; B5801,B1502 ; C8.C0302