CHAPTER IV

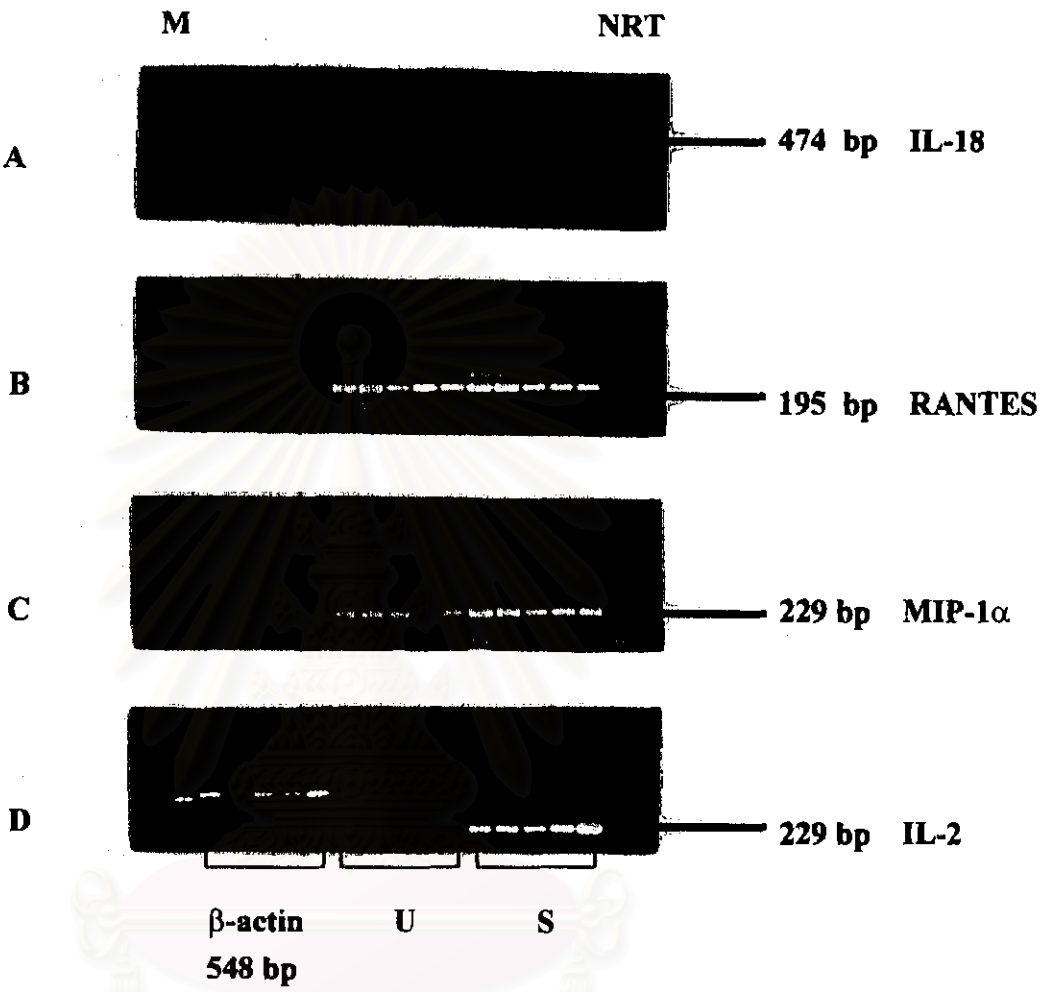
RESULTS

Part I Standardization of RT-PCR analysis for IL-2, IL-18, RANTES and MIP-1α mRNA production

PBMC from five normal donors were analysed for the production of IL-2, IL-18, RANTES and MIP-1α mRNA. The production of IL-18, RANTES and MIP-1α mRNA was detected in both unstimulated and stimulated PBMC (Figure 1 A, B, C). In contrast, IL-2 mRNA was not detected in unstimulated PBMC but it was induced by mitogenic stimulation with PHA (Figure 1 D).

Part II Reproducibility of RT-PCR amplification

As depicted in Figures 2 and 3, there was a positive relationship between cDNA concentrations and the yield of PCR products across the range of cDNA concentrations. Densitometric integration analysis demonstrated that RT-PCR amplification of 75, 37.5, 18.75 and 9.375 ng cDNA for a total of 33 cycles yielded a linear relationship with the PCR products. These results demonstrate a reproducibility and linearity of RT-PCR amplification.



M = marker, 100 bp DNA ladder

U = unstimulated PBMC

S = stimulated PBMC

NRT = non-reverse transcribed negative control

Figure 1. Standardization of RT-PCR analysis for cytokine and chemokine mRNA production in PBMC of HIV seronegative donors

Figure 2. Reproducibility of RT-PCR amplification A). β-actin and B). RANTES

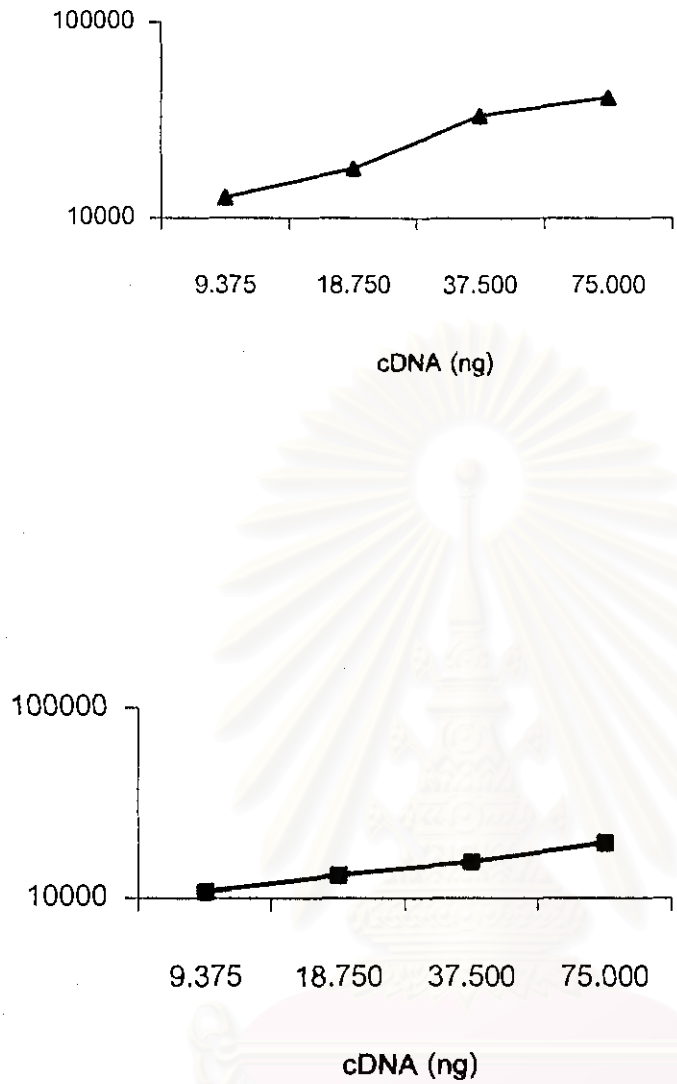


Figure 3. Densitometric integration analysis of A). β-actin and B). RANTES mRNA production

Part III Study of IL-2, IL-18, RANTES and MIP-1α mRNA production in PBMC of HIV-infected patients.

1. IL-2 mRNA expression in PBMC of HIV-infected patients received various dosages of IL-2 administration

PBMC of a total of 24 HIV-infected patients treated either with 1.5, 4.5 and 7.5 MIU bid of s.c. IL-2 were evaluated for IL-2 mRNA expression at the baseline and day 4 of the first cycle. Constitutive expression of IL-2 has never been detected in PBMC of patients before administration of IL-2. Significant differences of IL-2 mRNA expression was detected at day 4 between 1.5 and 4.5 MIU bid (P=0.04) and between 1.5 and 7.5 MIU bid dosing regimens (P=0.01). There was no significant difference between the 4.5 and 7.5 dosages (P=1.00). Table III and Figure 4 show the percentage of IL-2 mRNA expression depending on the respective dose of IL-2 administration, i.e., 12.5% in the 1.5 MIU bid group, 71% in the 4.5 MIU bid group and 77% in the 7.5 MIU bid group.

2. Comparison of IL-2 mRNA expression in HIV-infected patients treated with antiretrovirals alone and antiretrovirals plus s.c. IL-2

IL-2 mRNA expression in PBMC of the randomized 10 HIV-infected patients treated with antiretrovirals plus s.c. IL-2 was compared with those of 9 HIV-infected patients treated with antiretrovirals alone. Table IV shows the number of patients with IL-2 mRNA expression in both groups. At the baseline, mRNA expression of IL-2 was not detected in any PBMC samples obtained from either groups of patients. At week16, there was a significant difference between the proportion of IL-2 mRNA production in the antiretrovirals plus s.c. IL-2 group and the group treated with antiretrovirals alone, i.e. 50% vs 0%, respectively, P=0.03 (Figure 5).

Table III **Number of patients with IL-2 mRNA expression in PBMC after three different doses of IL-2 treatment at baseline and day 4 of cycle 1**

IL-2 doses (MIU bid)	No. of Patients with IL-2 mRNA/ No. of Patients tested		
	Day		
	baseline	4	P value
1.5	0/8	1/8 (12.5%)	-
4.5	0/7	5/7 (71.0%)	0.04*
7.5	0/9	7/9 (77.0%)	0.01**

* 1.5 vs 4.5 MIU bid at day 4

** 1.5 vs 7.5 MIU bid at day 4

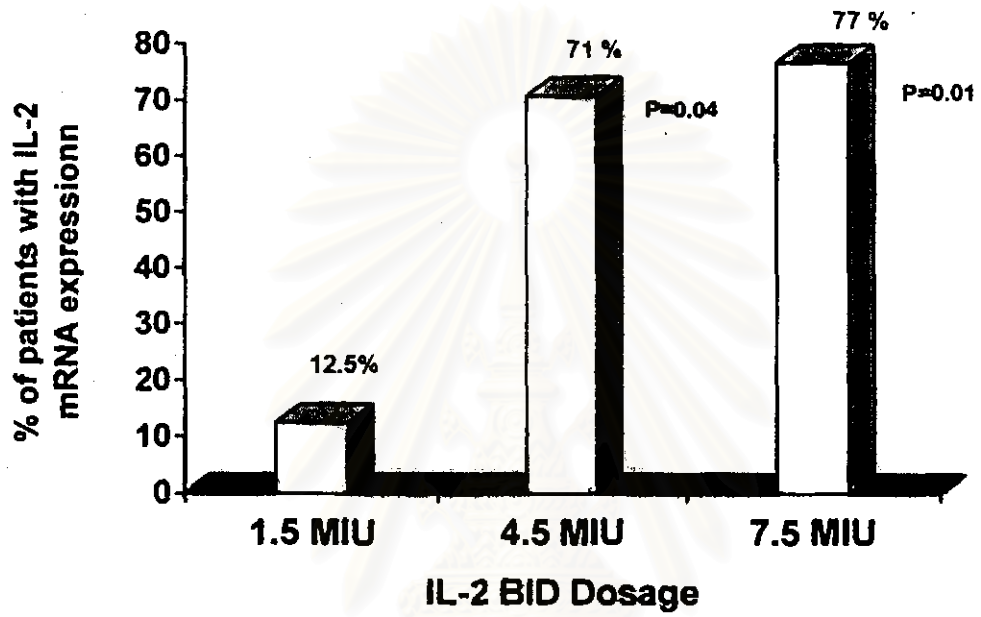


Figure 4. Dose-dependent IL-2 mRNA expression in PBMC of the ART plus IL-2 group at day 4 of cycle 1 of IL-2 therapy

Table IV **Number of patients with IL-2 mRNA expression in PBMC of ART and ART plus IL-2 group.**

Group	IL-2 mRNA expression	
	baseline	week 16
I. Antiretrovirals (ART) alone (n=9)[a]	0	0
II. Antiretrovirals (ART) plus IL-2 (n=10)[c]	0	5[b]

[a] Patient No. 26 was lost to follow-up

[b] P = 0.03; P refers to statistical significance of ART group vs ART plus IL-2 group at week 16

[c] 8 and 2 were received 7.5 and 4.5 MIU bid, respectively

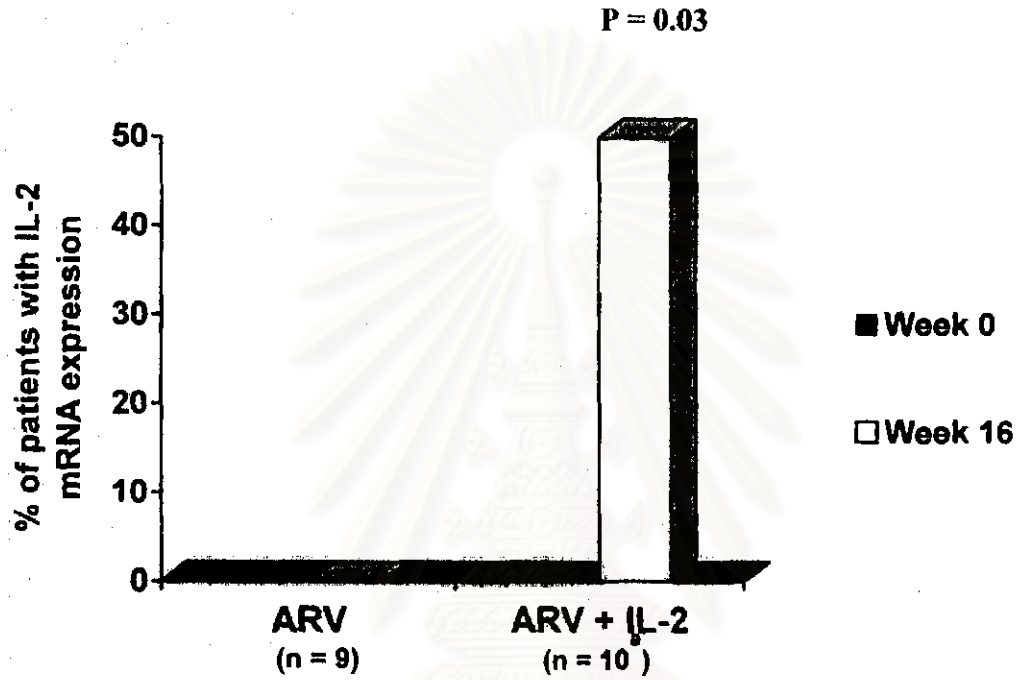


Figure 5. Percentage of patients with IL-2 mRNA expression in the ART group compared with the ART plus IL-2 group at baseline and week 16.

([a] 8 and 2 were received IL-2 7.5 and 4.5 MIU bid, respectively)

3. IL-2, IL-18, RANTES and MIP-1α mRNA production in PBMC of HIV-infected patients treated with antiretrovirals alone.

Figure 6 shows the mRNA production of cytokines and chemokines in the group treated with antiretrovirals alone at the baseline and week 8. IL-18, RANTES, MIP-1α mRNA production were detected in all PBMC samples obtained from patients at two points in time. In contrast, IL-2 mRNA production was not detected in all PBMC samples at the baseline but it was detected in 3 PBMC samples at week 8. However, the proportion of IL-18, RANTES, MIP-1α and IL-2 mRNA production was not significantly different between the baseline and week 8. ($P>0.05$) (Table V).

4. IL-2, IL-18, RANTES and MIP-1α mRNA production in PBMC of HIV-infected patients treated with antiretrovirals plus s.c. IL-2

Figure 7 shows the cytokine and chemokine mRNA production at the third cycle of IL-2 therapy. IL-18, RANTES, MIP-1α mRNA production were detected in all PBMC samples obtained from patients at precycle 3, day 4, and day 29 of the third cycle. In contrast, IL-2 mRNA production was detected in 5, 5, and 3 samples at precycle 3, day 4, and day 29 of the third cycle. There were no significant difference in the proportion of IL-18, RANTES, MIP-1α and IL-2 mRNA production between each point in time (precycle 3, day 4 and day 29) of the third cycle ($P>0.05$) (Table VI).

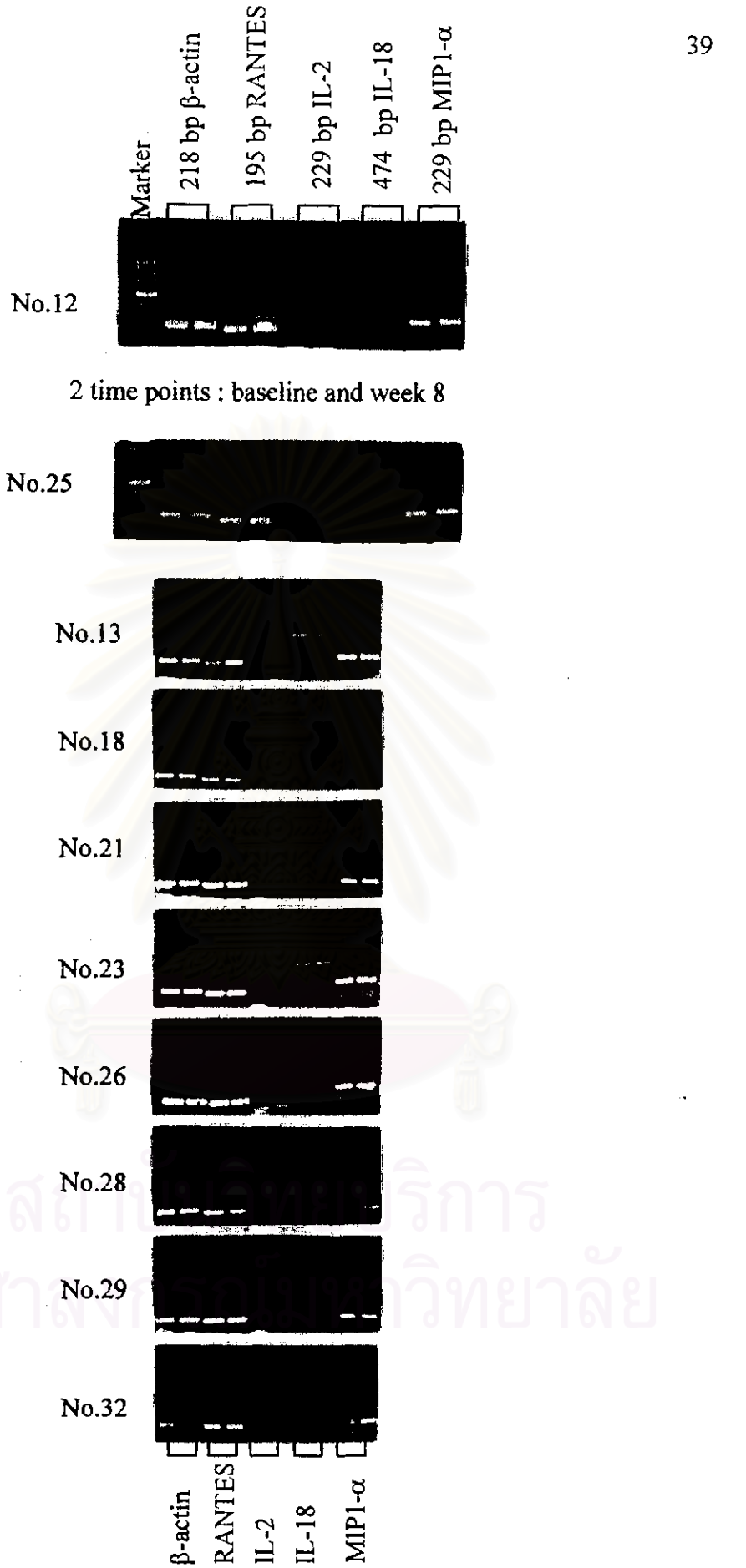


Figure 6. mRNA production of cytokines and chemokines in antiretroviral alone group at baseline and week 8

Table V Proportion of patients with cytokine and chemokine mRNA expression in PBMC of ART group

Cytokines/ Chemokines	Proportion of patients with cytokine/chemokine mRNA expression		
	baseline	week 8	P[a] value
Cytokines			
IL-2	0/10	3/10	>0.05
IL-18	10/10	10/10	>0.05
Chemokines			
RANTES	10/10	10/10	>0.05
MIP-1α	10/10	10/10	>0.05

[a] Statistical analysis by Fisher's exact test (using a statistical software package, SPSS version 6.0, 1993, SPSS Inc, USA)

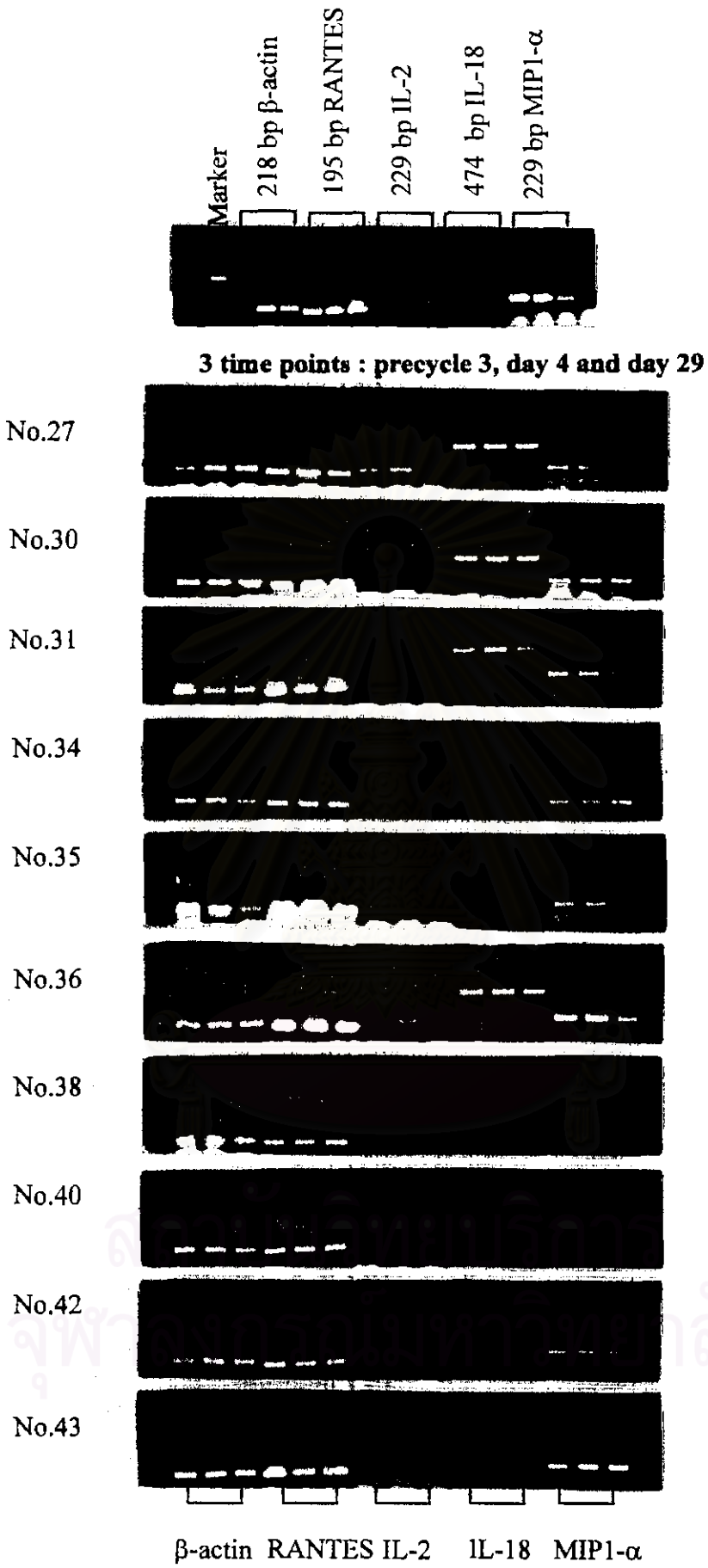


Figure 7. mRNA production of cytokines and chemokines in antiretroviral plus IL-2 group at the third cycle of IL-2 administration

Table VI **Proportion of patients with cytokine and chemokine mRNA expression in PBMC of the ART plus IL-2 group at the third cycle of IL-2 administration**

Cytokines/ Chemokines	Proportion of patients with cytokine/chemokine mRNA expression			
	precycle 3	day 4	day 29	P[a] value
Cytokines				
IL-2	5/10	5/10	3/10	>0.05
IL-18	10/10	10/10	10/10	>0.05
Chemokines				
RANTES	10/10	10/10	10/10	>0.05
MIP-1α	10/10	10/10	10/10	>0.05

[a] Statistical analysis by Fisher's exact test (using a statistical software package, SPSS version 6.0, 1993, SPSS Inc, USA)