Chapter IV

Results

1. CD4+ T cell counts in HIV seropositive patients

In HIV seropositve patients with CD4+ T cell counts ≥ 200 cells/μl (group B), median CD4+ T cell count was 397 cells/μl (range 220-979). Median CD4+ T cell count in group C, HIV seropositive patients with CD4+ T cell counts < 200 cells/μl , was 56 cells/μl (range 7-199). Individual CD4+ T cell counts were summarized in table 1.

Table 1. CD4+ T cell counts of HIV infected patients at the enrollment

HIV seropositive group B (CD4≥200 cells/μl)			HIV seropositive group C (CD4<200 cells/μl)		
Subject No.	Absolute CD4	% CD4	Subject No.	Absolute CD4	% CD4
B1	979	30	C1	199	11
B2	858	21	C2	177	11
B3	583	17	C3	135	7
B4	562	28	C4	127	8
B5	493	22	C5	120	12
B6	443	21	C6	111	5
B7	415	18	C7	105	8
B8	397	16	C8	56	7
B9	363	11	C9	49	7
B10	322	15	C10	34	3
B11	304	15	C11	31	4
B12	270	12	C12	29	4
B13	262	23	C13	12	3
B14	222	9	C14	12	2
B15	220	22	C15	7	2

2. RT-PCR Assay Validation

The linearity of amplification demonstrated by four of 2-fold dilution series (5 dilutions per each primer set) started at equal amount of 62.5 ng total RNA for PCR testing. The PCR amplification

products were seen in a certain difference at about 15 ng of total RNA or 1:8 diluted cDNA mixture for β-actin and IL-18 PCR respectively. For MIP-1α and RANTES, the certain difference of amplification was seen at about 8 ng of total RNA or 1:16 diluted cDNA mixture. (Figure 1)

β-actin	IL-18	MIP-1α	RANTES
548 bp	342 bp	229 bp	195 bp

Figure 1. Linearity of amplification testing for β-actin, IL-18, MIP-1α and RANTES (a two-fold dilution series, starting 1:2 of undiluted original cDNA)

3. RT-PCR Results

3.1 HIV seronegative individuals (group A)

Peripheral blood mononuclear cell (PBMC) samples from 14 out of 15 HIV seronegative subjects showed consitutive expression of IL-18 mRNA, whereas all of them showed detections of mRNA expression of MIP-1α and RANTES (Figure 2A and 2B)

Group A HIV seronegative individuals (control group)

Figure 2. Results of cytokine/chemokines RT-PCR from unstimulated PBMCs of 15 HIV seronegative donors. β-actin serves as a control. A). IL-18 (NRT = non-reverse transcribed control, MW = 100-bp molecular marker)

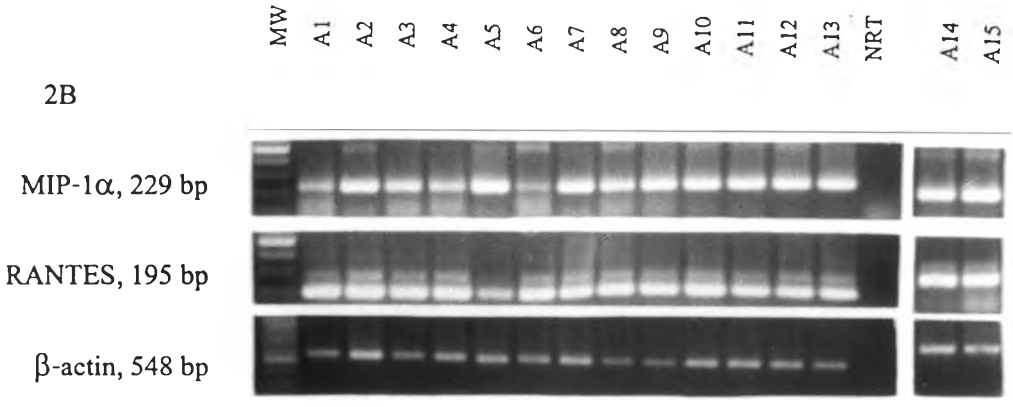

Figure 2. Results of cytokine/chemokines RT-PCR from unstimulated PBMCs of 15 HIV seronegative donors. β-actin serves as a control. B). MIP-1α and RANTES (NRT = non-reverse transcribed control, MW = 100-bp molecular marker) Note, A14 and A15 were run in gel separately.

3.2 HIV - infected asymptomatic patients with CD4+ T cell counts ≥ 200 cells/μl (group B)

IL-18, MIP-1α and RANTES mRNA were detected in unstimulated PBMC samples from all of the subjects in group B (Figure 3A and 3B)

Group B HIV-infected individuals with CD4+ T cell counts ≥ 200 cells/μl

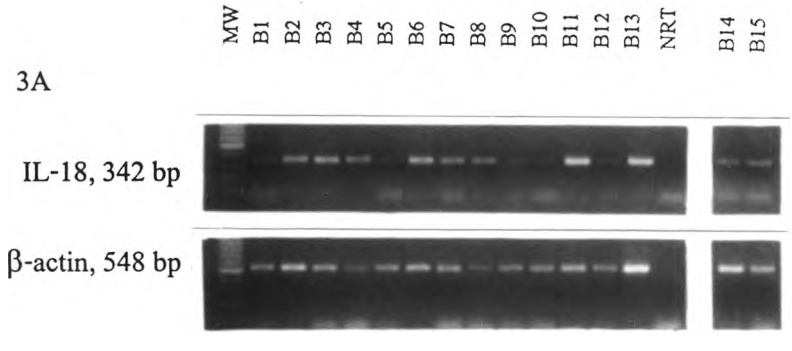

Figure 3. Results of cytokine/chemokines RT-PCR from unstimulated PBMCs of 15 HIV-infected individuals with CD4+ T cell counts ≥ 200 cells/μl. β-actin served as a control. A). IL-18 (NRT = non-reverse transcribed control, MW = 100-bp molecular marker)

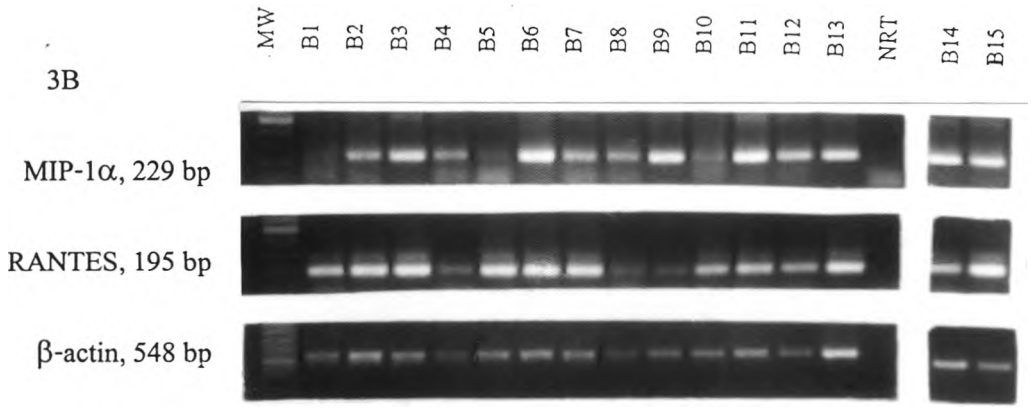

Figure 3. Results of cytokine/chemokines RT-PCR from unstimulated PBMCs of 15 HIV-infected individuals with CD4+ T cell counts ≥ 200 cells/μl. β-actin served as a control. B). MIP-1α and RANTES (NRT = non-reverse transcribed control, MW = 100-bp molecular marker) Note, B14 and B15 were run in gel separately.

3.3 HIV - infected symptomatic patients with CD4+ T cell counts < 200 cells/μl (group C)

All of the PBMC samples from HIV+ symptomatic patients with CD4+ T cell counts < 200 cells/μl showed IL-18, MIP-1α and RANTES mRNA expression, except in patients no. C15, there was no IL-18 mRNA detected signal. (Figure 4 A and 4B)

Group C HIV-infected patients with CD4+ T cell counts < 200 cells/μl

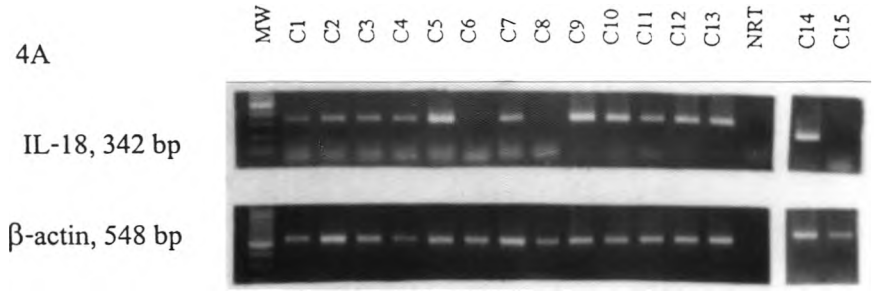

Figure 4. Results of cytokine/chemokines RT-PCR from unstimulated PBMCs of 15 HIV-infected patients with CD4+ T cell counts < 200 cells/μl. β-actin served as a control. A). IL-18 (NRT = non-reverse transcribed control, MW = 100-bp molecular marker)

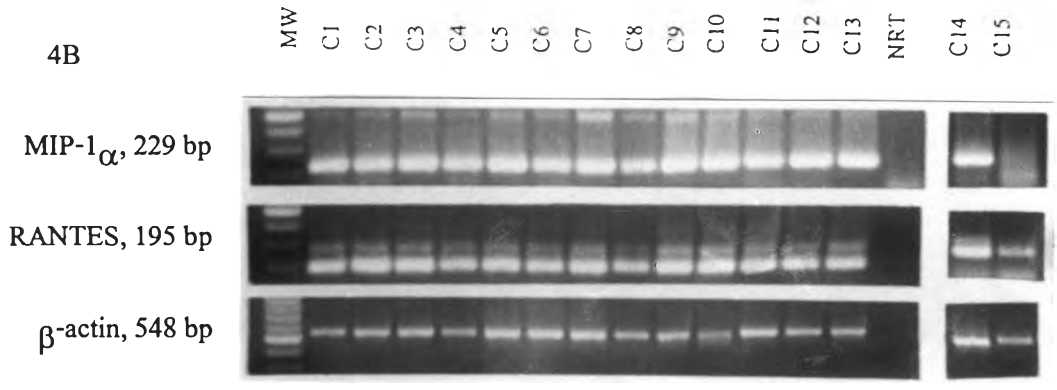

Figure 4. Results of cytokine/chemokines RT-PCR from unstimulated PBMCs of 15 HIV-infected patients with CD4+ T cell counts < 200 cells/μl. β-actin served as a control. B). MIP-1α and RANTES (NRT = non-reverse transcribed control, MW = 100-bp molecular marker) Note, C14 and C15 were run in gel separately.

4. Comparative Analysis

Almost all of the PBMC samples either from HIV-infected patients or non-infected controls showed mRNA expressions of IL-18, MIP-1α and RANTES. (Table 2)

Table 2. Numbers of subjects with cytokine/chemokines gene expression in PBMC samples from HIV seronegative group and the two subgroups of HIV seropositive persons

Study group	Subjects with cytokine/chemokines gene expression		
	IL-18	MIP-1α	RANTES
HIV control group (group A, n=15)	14	15	15
HIV seropositive group B (CD4 ≥ 200 cells/μl)(n=15)	15	15	15
HIV seropositive group C (CD4 < 200 cells/μl)(n=15)	14	15	15

Based on the qualitative RT-PCR results, there were no statistical differences of the proportion of subjects with mRNA expressions of IL-18, MIP-1α and RANTES among the groups.