CHAPTER IV

RESULTS

4.1 Activation of mononuclear cell populations in WF10 stimulated PBMC cultures

In this experiment, we assessed WF10 for its ability to induce cell activation. PBMC cultures were stimulated with different concentrations of WF10 (1:900,1:300,and 1:100 final dilution in cultures) for 24 h. As shown in Table 3 and Figure1, significant increase in number of CD4+CD69+, CD8+CD69+, CD56+CD69+, γδ+CD69+, and CD20+CD69+ was observed, especially in WF10 (1:300) stimulated PBMC cultures.

Table 3. The effect of WF10 on CD69 expression in PBMC cultures.

Population	Mean % positive ± S.E.(N=4)			
	Control	*WF10 ; 1:900*	*WF10 ; 1:300*	*WF10 ; 1:100*
CD4+CD69+	1.02 ± 0.23	2.78±0.36*	12.50 ± 3.62*	12.80± 2.92*
CD8+CD69+	4.63 ± 1.31	8.20±1.70	21.59 ± 5.22*	13.75 ± 4.30
CD56+ CD69+	23.48 ± 8.45	33.73±7.98*	67.11 ± 11.14*	64.83± 9.73*
γδ+CD69+	4.02 ± 1.41	8.17±3.20	45.58 ±4.17*	46.88± 8.67*
CD20+CD69+	10.65 ± 1.52	16.29±3.27	64.44 ± 4.39*	77.67± 1.82*

* $P<0.05$ compared with controls.

Data of each subject is presented in Appendix (Table 6, Table 7,Table 8,and Table9).

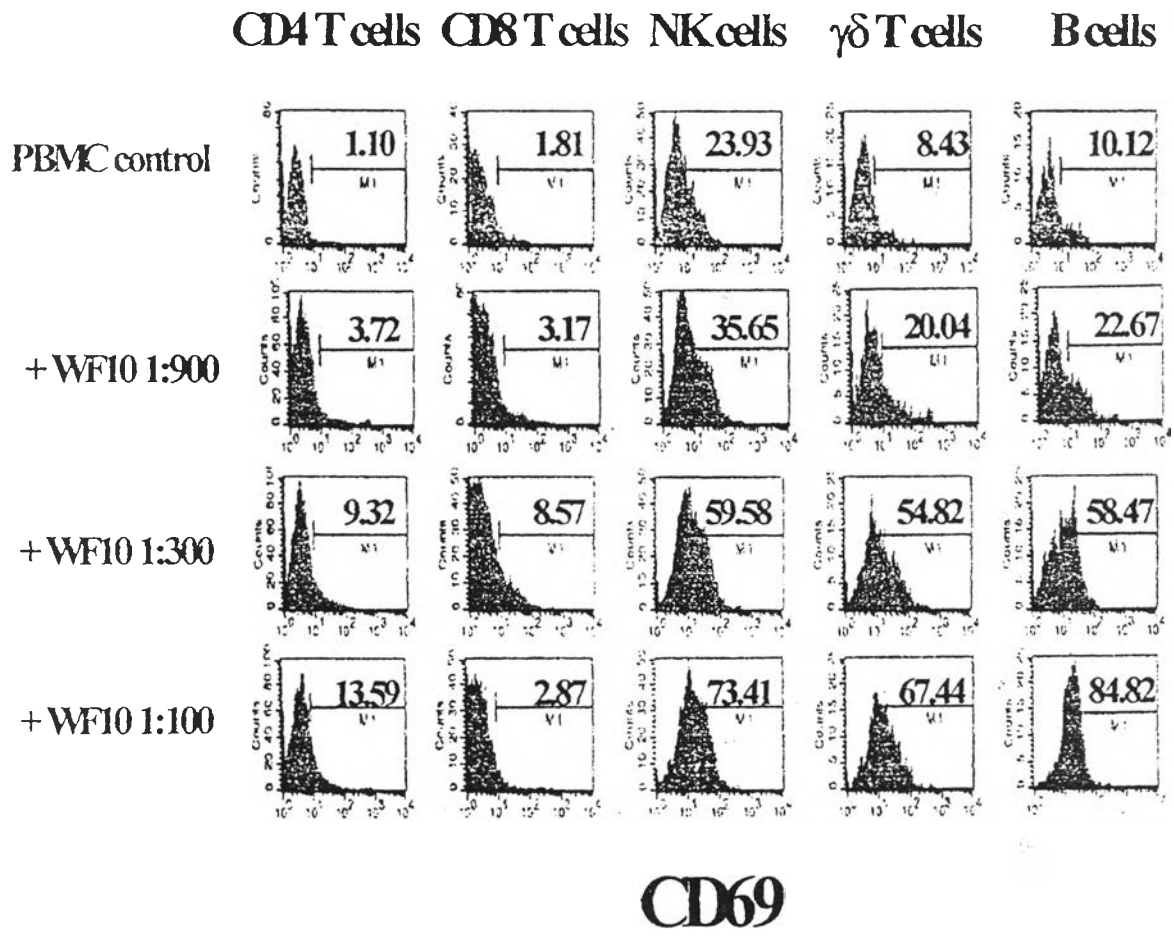

Figure 1. Representative histogram of CD69 expression on lymphocyte subsets in response to WF10 (n=4). PBMC were incubated with various concentrations of WF10 (1:900,1:300,and 1:100) for 24 h. Cells were then harvested, stained with mAbs cocktail combinations as described in Materials and Methods and then analysed for expression of CD69. Gates were set with isotype control antibodies. M1 regions consist of CD69+ cells. Numbers in each histogram represent % of cells that express CD69.

4.2 *Cytokines production by PBMC stimulated with WF10.*

We tested the ability of WF10 to induce cytokine production in PBMC cultures. Results in Figure 2 demonstrated that increased production of TNF-α, IL-12 (p40) and IFN-γ were observed in WF10 treated PBMC cultures when compared to the controls. WF10 induced moderate TNF-α, low IFN-γ and very minimal IL-12 production. TNF-α and IFN-γ production in WF10 treated PBMC cultures appeared to be dose dependent. The significant enhancement of TNF-α production was observed at all concentrations of WF10 while the significant enhancement of IFN-γ production was observed at 1:300 and 1:100 of WF10 as compared to the controls.

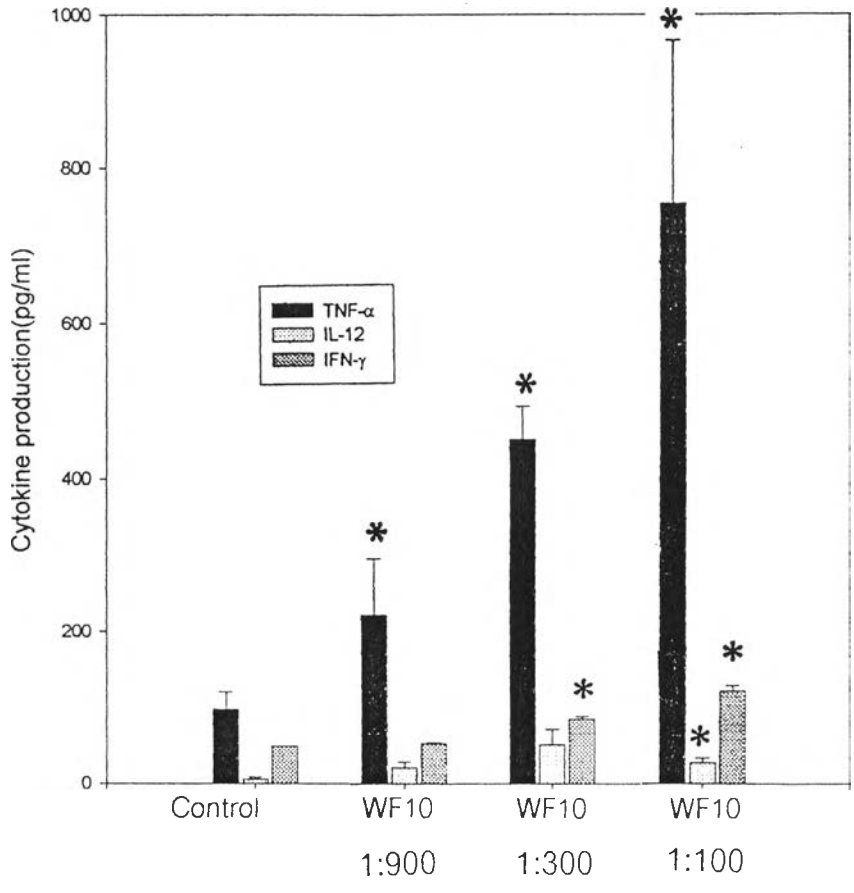

Figure 2. Cytokine production by WF10-treated PBMC. PBMC cultures were stimulated with WF10 (1:900, 1:300,and 1:100). Culture supernatant was collected after 24 hours of incubation and assessed for TNF-α, IL-12 (p40), and IFN-γ production by ELISA. Data of each subject is presented in Appendix (Table 10, Table 11, Table 12, and Table 13). * $P<0.05$ compared with controls.

4.3 *TNF-α production by PBMC stimulated with P. gingivalis and WF10*

We tested the ability of *P. gingivalis* and WF10 to induce TNF-α production in PBMC cultures (from two donors: subject E and F). *P. gingivalis* stimulated PBMC cultures to produce moderate amount of TNF-α in a dose-dependent manner (Figure 3). Statistical analysis (Table 4) showed that, in the absence of WF10, *P. gingivalis* at the concentration of 0.1 μg/ml had a significant effect on TNF-α stimulation when compared to the controls. WF10 alone at a final concentration of 1:100 in culture significantly induced TNF-α production. Interestingly, when combined WF10 with *P. gingivalis*, the combination appeared to have synergistic effect on TNF-α production. However, When compared to *P. gingivalis* alone, the combination of WF10 and *P. gingivalis* at 0.01 μg/ml, not other concentrations, significantly induced TNF-α production. Furthermore, TNF-α production was also significantly increased by WF10 combined with 0.001 μg/ml or 10 μg/ml *P. gingivalis* when compared with that of WF10 alone. Although variation of the amount of TNF-α production existed between two subjects as seen by high S.E. (Table 4), similar patterns of TNF-α production in PBMC cultures were observed between the two subjects (Figure 3). Data of each subject is presented in Appendix (Table 14 and Table 15).

Table 4. Mean ± S.E. TNF-α production (pg/ml) by PBMC stimulated with *P. gingivalis* and WF10 (N=2).

Sample	*P. gingivalis* concentrations (μg/ml)					
	Control (No *P. gingivalis*)	*P. gingivalis* 0.001	*P. gingivalis* 0.01	*P. gingivalis* 0.1	*P. gingivalis* 1	*P. gingivalis* 10
No WF10	76.60 ± 46.75	79.52 ± 45.47	631.02 ± 483.57	2141.74 ± 80.80 *	7486.02 ± 1314.24	9636.81 ± 2840.74
With WF10 (1:100 final dilution)	881.81 ± 23.45 #	926.64 ± 148.86 #	2133.21 ± 77.20 *	10102.07 ± 1869.23	13222.32 ± 2762.85	17203.87 ± 3986.61 #

* $P<0.05$ compared with controls (no *P. gingivalis*).

# $P<0.05$ compared between *P. gingivalis* alone (no WF10) at each concentration.

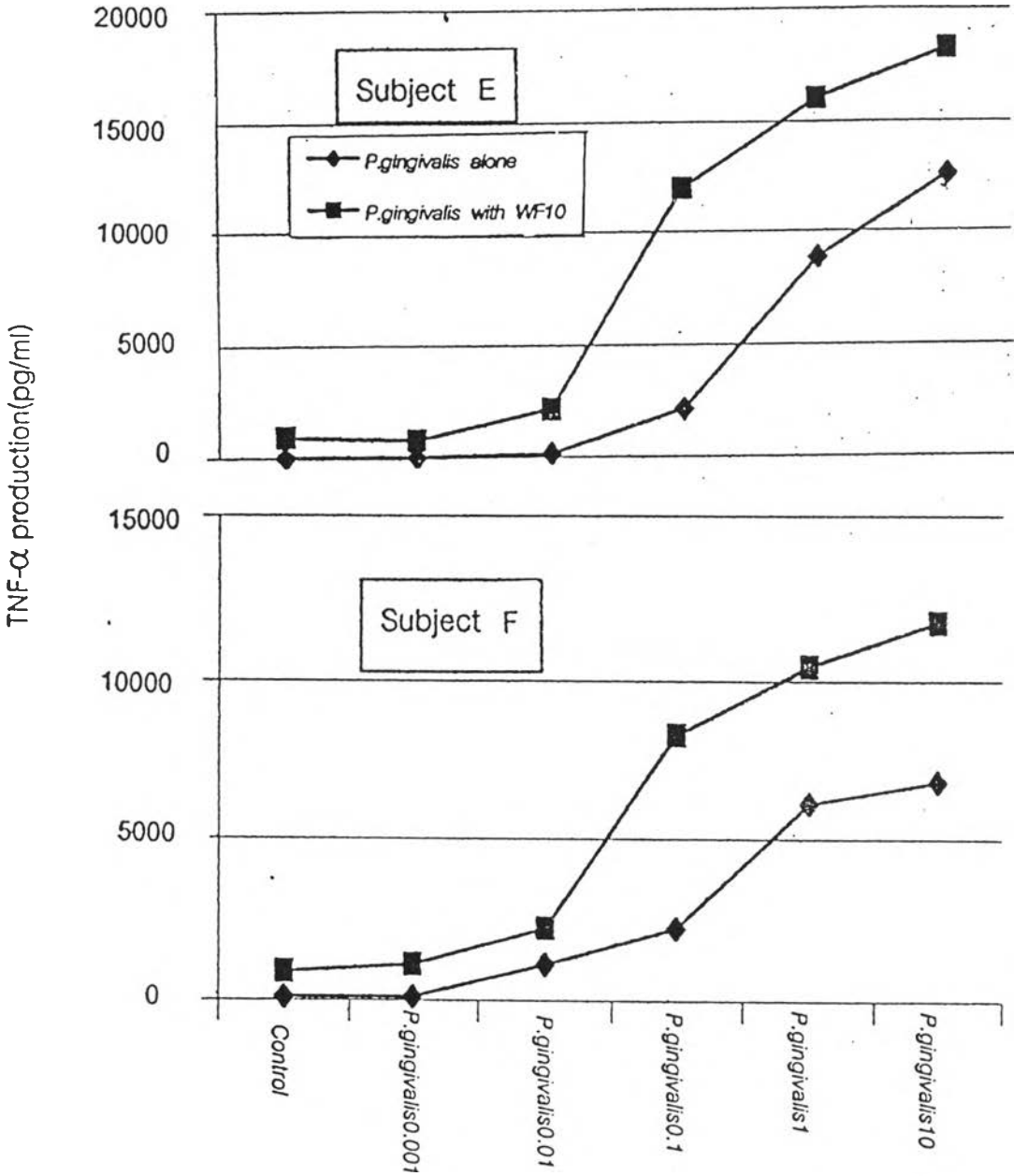

Figure 3. TNF-α production in PBMC cultures (from 2 donors: subject E and F) stimulated with *P. gingivalis* and WF10. PBMC cultures were stimulated with different concentrations of *P. gingivalis* or combined with WF10(1:100) for 24 h. Supernatants were harvested and then assayed for TNF-α production by ELISA. .

4.4 IL-1β production by PBMC stimulated with P. gingivalis and WF10

We tested the ability of *P. gingivalis* and WF10 to induce IL-1β production in PBMC cultures (from two donors: subject G and H). Stimulation with *P. gingivalis* apparently led to the production of IL-1β in PBMC cultures, in a dose dependent manner (Figure 4). Statistical analysis (Table 5) showed that, in the absence of WF10, *P. gingivalis* at all concentrations did not have a significant effect on IL-1β production when compared to the controls. This may be due to high S.E.. In contrast to the synergistic effect on TNF-α production, the combination of WF10 and *P. gingivalis* appeared to down-regulated IL-1β production. When compared to WF10 alone, a statistically significant decrease in IL-1β production was observed in the culture of WF10 with 0.1 μg/ml *P. gingivalis* and not other concentrations. Furthermore, when compared to *P. gingivalis* alone, the combination of WF10 and *P. gingivalis* at 1 μg/ml gave a statistically significant decrease in IL-1β production. Similar patterns of IL-1β production in PBMC cultures were observed between two subjects. (Figure 4). Data of each subject is presented in Appendix (Table 16 and Table 17).

Table 5. Mean ± S.E. IL-1β production (pg/ml) by PBMC stimulated with P. *gingivalis* and WF10 (N=2).

Sample	Control (No *P. gingivalis*)	*P. gingivalis* concentrations (μg/ml)				
		P. gingivalis 0.001	*P. gingivalis* 0.01	*P. gingivalis* 0.1	*P. gingivalis* 1	*P. gingivalis* 10
No WF10	33.56 ± 18.58	34.73 ± 16.24	542.47 ± 355.78	4175.41 ± 1165.27	9409.66 ± 322.76	17203.87 ± 3986.61
With WF10 (1:100 final dilution)	17.90 ± 7.90	26.13 ± 10.64	226.00 ± 119.37	806.34 ± 83.45 *	2411.76 ± 523.40 #	2911.86 ± 842.61

* $P<0.05$ compared with controls (WF10 alone)

# $P<0.05$ compared with *P. gingivalis* alone (no WF10) at each bacterial concentration.

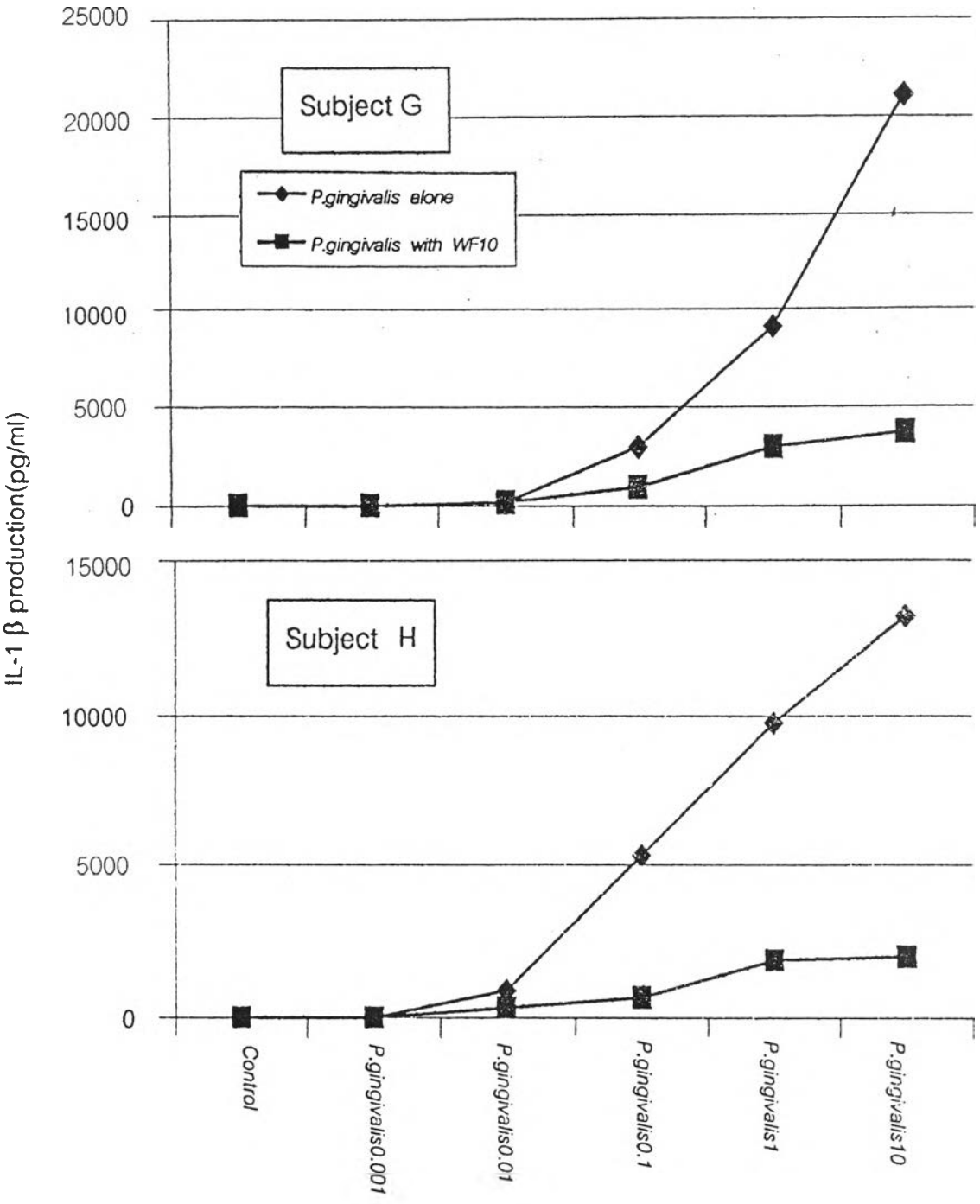

Figure 4. IL-1β production in PBMC cultures (from 2 donors: subject G and H) stimulated with *P. gingivalis* and WF10. Supernatant from PBMC cultures stimulated with different concentrations of *P. gingivalis* alone or combined with WF10 (1:100) for 24 h. were assessed for IL-1β production.