CHAPTER 5

Conclusions and Discussion

The present study has demonstrated that human T-cell lines with specific reactivity for *P. gingivalis* could be established from peripheral blood of periodontitis patients. Even though, both lines were derived from the two patients manifesting the similar level of disease activity and were maintained under the same culture conditions up to 6-8 weeks, the FACs analysis showed the difference in the proportions of CD4+ and CD8+ cells between the two TCLs. While one line (CC-TCL) was dominated by the CD4+ cells (67%), the other line was dominated by the CD8+ cells (73%). Despite different phenotypes, both *P. gingivalis* responsive TCLs secreted IFN-γ but not IL-4 after antigen stimulation, thus indicating a prominent type 1 T-cell response.

The appropriated radiation dose in this experiment was 30 Gray which could be seen in low proliferative response in the culture without *P. gingivalis* or even with *P. gingivalis* or PHA both on day 3 and day 7 comparing with non-radiated culture. This indicates the ability of radiation to inhibit growth of LCLs.

A very early study on the blastogenic response of peripheral blood lymphocytes from severe periodontitis patients to periodontopathic bacterial sonicates demonstrated a depressed response (Ivanyi and Lehner, 1971) although

other studies on responses to *P. gingivalis* had reported conflicting results (Stashenko et al., 1983; Shenker and Slots, 1989). Recently, Gemmell et al. (1995) showed a trend towards a lower proliferative response to *P. gingivalis* by PBMC from adult periodontitis subjects as compared with those from gingivitis subjects. In the present study both peripheral blood TCLs from severe periodontitis appeared to proliferate well in the presence of whole cell heat-killed bacterium with a comparable proliferative response to PHA except for the CC-TCL on Day 28. In addition the specificity for the antigen originally used for stimulation was maintained, and the increase in responsiveness of both TCLs to the antigen was observed over the time course of the culture period. This is in contrast to the former study (Gemmell et al., 1995) in which the stimulation was done with the outer membrane antigens of *P. gingivalis*, both gingivitis and periodontitis derived peripheral blood TCLs proliferated very slowly, and the level of response was virtually not much different from the unstimulated cultures. This may be due to the differences in the nature of the antigens, i.e. the outer membrane and the whole cell of *P. gingivalis*. Besides that, those two patients in the present study seemed to manifest a more advanced periodontal disease stage than the other study since they were diagnosed as generalized severe adult periodontitis with a few teeth needed to be extracted according to extensive loss of periodontal attachment and bone support.

As expected that TCLs consisted of heterogenous CD4+ cells and CD8+ subsets. The majority of T-cells in each TCL from periodontitis subjects were

different with regards to their surface phenotypes. While one line (CC-TCL) was dominated by CD4+ cells and the other line (SA-TCL) was dominated by CD8+ cells. Therefore, the tissue culture procedure does not appear to select for cells with specific phenotypes. On the other hand, it was thought that in healthy periodontal subjects, CD8+ T-cells may be preferentially responsive to *P. gingivalis* or the organism may exert a suppressive effect on the CD4+ T-cell population. This came from the study of peripheral blood TCLs reactive to *P. gingivalis* and *F. nucleatum* in healthy periodontal subjects (Ishii et al., 1992). They found that *P. gingivalis* reactive TCLs presented the same proportion of both T-cell subsets, CD4 and CD8, while the other bacterium, *F. nucleatum*, reactive TCLs showed a predominance of the CD4+ subset. The results, however, suggest that TCLs derived from healthy and diseased subjects may consist of different specific T-cells.

The CD4:CD8 ratios of the two peripheral blood TCLs reactive to *P. gingivalis* in this study were 1.9 and 0.2. As expected T-cell lines were polyclonal T-cells consisting of mixed population of CD4 and CD8. However, such ratios did not support previous studies where it was frequently found to be suppressed in patients with progressive disease (Taubman et al., 1984; Stoufi et al., 1988; Gemmell et al., 1994b; Gemmell et al., 1996). Although conflicting results of cell surface phenotype seemed to exist, it was demonstrated that both *P. gingivalis* responsive TCLs produced the same sort of cytokine, i.e. IFN-γ and none of IL-4. This again agrees with the concept of Type 1 and Type 2 T-cells where the division of functional T-cell subset depend on cytokine profiles instead of their phenotypes.

IFN-γ was detected in both TCL culture, CC and SA after 6 hr., 1 day, 3 day incubation period with *P. gingivalis*. However, it could be noted that in unstimulated culture, certain amount of IFN-γ was also present. This may result from previous exposure of the cells to *P. gingivalis* during chronic stimulation. On the other hand, IL-4 was not detected in both TCL culture in any incubation periods.

The cytokine results of the present study indicate the prominent Type 1 T-cells in both *P. gingivalis* cell lines. This does not support the previous model proposed by Seymour et al. (1993) where they hypothesized that Type 1 T-cell may be associated with non-susceptible subjects or a stable lesion and Type 2 T-cell may be associated with susceptible subjects or progressive lesion. Recently, this group investigated cytokine profiles of *P. gingivalis* reactive T lymphocyte lines and clones derived from *P. gingivalis* infected subjects, one gingivitis and one periodontitis. They found that peripheral blood T-cell lines from each patient produced both intracytoplasmic IL-4 and IFN-γ as measured by FACs analysis. There was very little difference in the percentage of IFN-γ producing CD4 and CD8 cells in both peripheral blood TCLs. This was further confirmed by the mRNA expression for both cytokines in the TCL from periodontitis patients which were detected by reverse transcriptase polymerase chain reaction (RT-PCR). Interestingly, the stronger band of IFN-γ was noticed when compared with the IL-4. Hence it seemed that *P. gingivalis* specific cells in their T-cell lines were the mixture of Type 1 and Type 2 T-cell or Th0. However, the clones established from this line

produced IL-4, not IFN-γ and they were all CD4+ cells. It might be the cloning procedure used in their study led to a preferential growth of some clones or favored the proliferation of CD4+ clones since CD4/CD8 ratio of the clones did not completely match the CD4/CD8 ratio (0.4) found in the TCL (Gemmell et al., 1996). Furthermore, the results from the gingival tissue-derived T-cell clones specific to *P. gingivalis* also showed the same trend as those peripheral blood T-cell clones. Intracytoplasmic IFN-γ and IL-4 was present in all the gingival tissue clones but they did not express mRNA for either cytokine (Gemmell et al., 1995). This might indicate the appropriate time for detection of mRNA expression is very crucial. In another study by Wassenarr et al. (1995), one *P. gingivalis* specific gingival T-cell clone derived from periodontitis subject was established. This clone did not produced IFN-γ or IL-4 as measured by ELISA. As could be seen that the cytokine data of antigen specific T-cells in periodontal disease were still inconclusive. It seemed that the method of cytokine detection was very crucial and further research was obviously required.

A few recent studies investigated cytokine profiles of gingival mononuclear cells. Fujihashi et al. (1993) had shown that gingival mononuclear cells from adult periodontitis patients produced IL-4 and IL-5 but not IL-2. This group then focused on Type 1 and Type 2 cytokine by CD4+ cells. They found that the gingival CD4+ T-cells associated with periodontitis expressed mRNA for IFN-γ, IL-6, IL-13 and in some instances IL-10 but not for IL-2, IL-4 and IL-5 (Fujihashi et al., 1996). A further study by Takeichi et al. (1994) demonstrated that 21.4% of CD4+ T-cells

isolated from the gingival tissues of adult periodontitis subjects expressed IL-2 mRNA, 64.3% expressed IFN-γ and 35.7% expressed IL-5 message. Similarly, 14.3% CD8+ T-cells extracted from these tissues were positive for IL-2, 71.4% were positive for IFN-γ and 57.1% expressed IL-5 mRNA. These results showed that a high percentage of both CD4+ and CD8+ cells extracted from adult periodontitis tissues expressed IFN-γ mRNA, overall, these two studies suggested the presence of the mixture of Type 1 and Type 2 T-cells or Th0 cells in periodontal lesion. However, it should be taken into consideration that these gingival mononuclear cells were the repertoire of antigen specific T-cells with different specificity to certain periodontopathic bacteria. Each antigen specific T-cell might participate in the host immune response to the relevant pathogen and contribute different types of cytokine in periodontal lesion. For example *P. gingivalis* specific cells in the present study produced IFN-γ. In addition, rat T-cell clones specific for *P. gingivalis* secreted IL-2 and IFN-γ (Katz et al., 1990), indicative of Type1 T-cells, whereas Eastcott et al. (1990) found that the rat T-cell clones specific to *Actinobacillus actinomycetemcomitans* showed an undetectable level of IL-2 activity and a failure to transfer DTH, suggestive of Type 2 T-cells. Therefore, the mixture of Type 1 and Type 2 T-cell cytokines reported in the studies of gingival cell is not surprising.

The results of the present study have shown that IFN-γ producing T-cells were present in *P. gingivalis* responsive TCLs. Continual production of IFN-γ by *P. gingivalis* specific T-cells may contribute to the persistent accumulation of

monocytes and macrophages, since this cytokine has been shown to up-regulate monocyte/macrophage differentiation and function. IL-1 or osteoclast activating factor has been shown to produced by monocyte/macrophage, and in progressive periodontal disease this cytokine was found to be elevated. In addition, IFN-γ was also shown to down-regulate the function of Type 2 T-cell. The persistent and strong Type 1 responses to *P. gingivalis* which are represented by production of IFN-γ may contribute to the complex network of pathogenesis of chronic inflammatory periodontal disease. Obviously future research into the role of this antigen specific T-cells in the disease would be required.