CHAPTER 5

Discussion and Conclusion

Inflamed gingival tissue has been characterized by infiltration of activated immune cells including T-cells and B-cells. It was suggested that T-cells infiltrated into the inflamed sites at the onset of inflammation, and that B-cells infiltrated later than T-cells (Matsuo et al., 1996). Based on the predominance of cell types at the lesion, periodontal lesions can be divided into 2 stages: a T-cell lesion which is also termed a " stable lesion" and a B-cell lesion which is also termed a "progressive lesion" (Seymour, 1991; Seymour, et al., 1993). Our results showed that B-cells were dominated cells in periodontitis lesions, and the majority of immune cells were activated cells (Table 2 and Figure1). This is in support of previous studies in peripheral blood as well as in gingival tissues of periodontitis patients (Afar et al., 1992; Gemmell and Seymour, 1991). It should be noted that the percentages of activated cells in our studies were higher than those of previous reports and this may be due to the measurement of CD69 expression which represents an early activation marker instead of measuring other markers; CD23, CD25 or HLA-DR as in other studies. Recently CD69 has been widely studied and shown to be a very sensitive activation marker (Santis et al., 1995), however, the presence of this marker among a variety of activated cell populations in periodontal area has never been assessed.

A large number of many activated T-cells, B-cells and other types of immune cells were known to infiltrate into inflamed gingival tissues, however, the role of these infiltrates remains unclear. Understanding the mechanisms of

how infiltrated mononuclear cells become activated could be essential to prevent / reduce immune-mediated tissue destruction in periodontitis. In this study, we used an *in vitro* model to study the activation of immune cells by periodontopathic bacteria, *P. gingivalis*.

PBMC cultures derived from healthy donors incubated with sonicated extracts of *P. gingivalis* led to a dose-dependent activation of different lymphocyte subpopulations, as monitored by the expression of an early activation antigen (CD69). Large increase in number of CD69+ cells was consistently observed in B-cells, NK cells and γδ T-cells, and less to a degree in αβ T-cells (Figure 2). When flow cytometric sorted cells were used, it is found that only B-cells but not αβ T-cells, γδ T-cells or NK cells were directly activated to increase expression of CD69 in response to *P. gingivalis* (Figure 3). Taken together, the data suggest that the activation of αβ T-cells, γδ T-cells and NK cells in *P. gingivalis*-stimulated PBMC cultures is not directly mediated by bacterial products. It is possible that soluble mediator(s) produced in *P. gingivalis*-stimulated PBMC cultures were responsible for activation of these cells. Further study is required to investigate soluble factor(s) involved T-cell and NK cell activation.

Hyperactivation of B-cells or polyclonal B-cell activation is a hallmark feature associated with progressive lesions of adult periodontitis (Bick et al., 1981; Carpenter et al., 1984; Tew et al., 1989; Ishikawa et al., 1997). High level production of B-cell regulatory cytokines, IL-5 and IL-6 have been detected in gingival mononuclear cell cultures of adult periodontitis patients and suggested to be critical mediators in the induction of hyperresponsiveness of B-cells (Fujihashi et al., 1993b). To further elucidate

the role of other B-cell regulatory cytokines, we assessed the production of IL-10, IL-12 and IL-15 in *P. gingivalis*-stimulated PBMC cultures. Large amount of IL-10 was detected in culture supernatants, while IL-12 and IL-15 were not detected. Our findings support recent studies by Yamazaki et al. (1997) and Aramaki et al. (in press) which showed the increased level of IL-10 mRNA and IL-10 protein in inflamed gingival tissues. The cellular source of IL-10 detected in gingival tissues is not clear. IL-10 producing T-cells have been detected in gingival tissues (Fujihashi et al., 1996). High level productions of IL-10 have been detected from clones of T-cells derived from periodontitis lesion (Mahanonda, R.; personal communication). Our *in vitro* model indicates that monocytes are the major source of IL-10 in *P. gingivalis*-stimulated PBMC cultures. The production was rapid and peaked within 24 hours. B-cells and αβ T-cells did not contribute to the *P. gingivalis* induced IL-10 production.

IL-10 was initially described as a cytokine synthesis inhibition factor (Moore et al., 1990; De Waal Malefyt et al., 1991). Later investigations have suggested the critical role of IL-10 in B-cell proliferation and antibody production (Rousset et al., 1991; 1992; Mosmann, 1994). High levels of IL-10 have been linked to the pathogenesis of autoimmune diseases including systemic lupus erythematosus, Sjogren's syndrome and rheumatoid arthritis (Llorente et al., 1993; 1994). In periodontitis, Stein and his colleagues (1997) have recently suggested that increased IL-10 production leads to expansion of CD5+ B-cells and enhances production of autoantibody which directs against gingival tissue components. The role of IL-10 to suppress cell-mediated immune response in periodontitis is unknown. Recent data have shown that IL-10 inhibits neutrophil phagocytic and bactericidal activity

(Laichalk et al., 1996). Thus, increased production of IL-10 in gingival tissue may suppress phagocytic cell function against periodontopathic bacteria and lead to disease development. In these experiments, we have shown that stimulation of purified B-cells with *P. gingivalis* sonicated extracts resulted in moderate B-cell proliferation. The proliferative response of B-cells was found to be significantly enhanced in the presence of IL-10. The results strongly support the role of IL-10 in B-cell activation and more important, it provides the mechanism underlying the predominance of B-cells in periodontitis lesions. In response to *P. gingivalis* infection, host immune cells, possibly monocytes and antigen reactive T-cells produce IL-10. We hypothesize that the produced IL-10 may inturn augment the activation of *P. gingivalis* (LPS) stimulated B-cells, resulting in rapid proliferation of B-cells and production of autoantibody. Furthermore, IL-10 may suppress defensive function of phagocytic cells and result in more susceptibility to infection.

The role of hyperresponsiveness of B-cells or polyclonal B-cell activation in periodontitis remains elusive. It is perceived as a non-protective reaction and rather contributes to the pathogenesis of the disease. Anti-collagen antibody has been consistently detected and suggested to be involved in gingival tissue destruction (Ftis et al., 1986; Hirsch et al., 1988b; Jonsson et al., 1991). In addition to its well known role in antibody production, B-cells also have the determinative role as antigen presenting cells (APCs) in the generation of T-cell mediated immune response (Hodgkin et al., 1995). Recent studies have shown that B-cells are critical in the pathogenesis of T-cell mediated autoimmune diseases (Serreze et al., 1996; Noorchashm et al., 1997). They serve as APCs in the generation of autoreactive T-cells involved in autoimmune response (Falcone et al., 1998).

Collagen-specific T-cell clones have been established from gingival tissues of chronic adult periodontitis patients (Wassenaar et al., 1995). Lymphocytes isolated from periodontal lesions exhibit high degree of cytotoxicity against fibroblast (O'Neill et al., 1982). Taken together, the data suggested the presence of autoreactive T-cells in the inflamed tissues. Development of Th subset is controlled at level of antigen presenting cells. Dendritic cells produce IL-12 and thus, they influence the development of Th1 cells meanwhile, B-cells influence the development of Th2 cells since they fail to produce IL-12 (Guery et al., 1997). A strong Th2 response has been detected in inflamed human gingival tissues (Fujihashi et al., 1996). Although B-cells contribute the majority of infiltrated immune cells in periodontitis lesion, there is no study so far to analyze the function of tissue infiltrated B-cells as APCs. The speculative role of B-cells to serve as APCs to drive Th2 cell development and to promote autoreactive T-cell mediated tissue destruction in periodontitis is provocative and more studies are needed to test this concept.

In conclusion, we have shown that stimulation of PBMC isolated from naive donors with sonicated extracts of *P. gingivalis* leads to activation of B- cells, NK cells, γδ and αβ T-cells. *P. gingivalis* sonicated extracts directly stimulate B-cells but not NK, γδ and αβ T-cells to express CD69. IL-10 production is detected in *P. gingivalis*-stimulated PBMC cultures, upon exposure of B-cells with *P. gingivalis* sonicated extracts and IL-10, proliferative response of B-cells is significantly increased. IL-10 may prove to be the critical cytokine involved in polyclonal B-cell activation associated with periodontal disease.