CHAPTER 1

Introduction

1.1 *Background of Present Study*

Periodontal disease is chronic inflammation of tooth supporting structures including gingiva, connective tissue and alveolar bone which caused by a complex interaction between host defenses and microorganisms in dental plaque (Sosroseno and Herminajeng, 1995). Periodontal disease could be clinically defined into two distinct groups, gingivitis and periodontitis (Caton,1989). Gingivitis is relatively stable. It is clinically characterized by increased redness, swelling and bleeding of the gingiva during brushing or probing. Without any periodontal treatment, the lesion may be confined to the marginal tissues and does not endanger the life of the dentition (Hirschfeld and Wasserman, 1978; McFall, 1982). This lesion has been termed the " stable lesion " (Seymour et al., 1979a). On the other hand, periodontitis, an advanced form, shows the clinical appearance of deepened gingival sulcus and loosing of the tooth due to periodontal tissue destruction. This periodontal breakdown appears to be cyclical or periodic (Goodson et al., 1982). This lesion has been termed the " progressive lesion " (Seymour et al., 1979a).

Etiology: Microbial aspects of periodontal disease

For many years, it has been recognized that periodontal diseases are caused by accumulation of bacteria on the surface of the tooth and beneath the gingiva (Socransky and Haffajee, 1992). At healthy and gingivitis sites,

composition of microbial plaque is quite similar and the majority is Gram positive facultative bacteria, such as *Streptococci* and *Actinomyces* (Slots, 1977a). It is known that gingival inflammation often related to the amount of dental plaque but not to the specific pathogens. In contrast, plaque associated with periodontitis appears to be a specific group of bacteria or critical pathogens in mixed infection. Gram negative anaerobes such as *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Bacteroides forsythus*, *Prevotella intermedia* and *Treponema denticola* are often found in subgingival plaque of periodontitis patients (Slots, 1977b; Dzink et al., 1988; Simonson et al., 1988). These pathogens have been implicated in the initiation and progression of different forms of periodontitis. *Porphyromonas gingivalis* is a Gram negative, anaerobic, non motile, asaccharolytic rod. It is a member of the much investigated black-pigmented *Bacteroides* group which have long history associated with periodontal disease. These species produce an unusually large array of virulent factors such as collagenase, an array of proteases (including those that destroy immunoglobulins), endotoxin, fatty acids, NH_3, H_2S, indole etc. (Macdonald et al., 1956; 1963). *P. gingivalis* is more frequently detected in destructive forms of periodontal disease but uncommon and in low numbers in health or gingivitis. The species have been shown to be reduced in successfully treated sites but is commonly encountered in sites that exhibit recurrence of disease post-therapy (Bragd et al., 1987; Van Winkelhoff et al., 1988). Interestingly, *P. gingivalis* has been shown to be able to invade human gingival epithelial cells *in vitro* (Duncan et al., 1993). This intracellular shelter would make these microorganisms possible to escape not only from host defense mechanisms but also from mechanical removal out of periodontal pocket via scaling and root planing.

Pathogenesis: Host response to plaque microorganisms

The pioneer study of Brandtzaeg (1973) showed large numbers of immunoglobulin-producing plasma cells in periodontitis lesions. Subsequent immunohistological studies using a range of enzymes and surface antigen markers have confirmed the characteristic of a B-cell and plasma cell dominated lesion in progressive periodontal disease (Seymour and Greenspan, 1979; Seymour et al., 1979b; Matsuo et al., 1996). On the contrary, the gingivally confined lesion or the stable lesion, is characterized by a predominance of T-cells. Hence, with disease progression, the change from T-cell dominated lesion to one consisting of a large numbers of B-cells were observed (Taubman et al., 1988).

Most work on immunopathogenesis of periodontal disease has, however, been focused on immunoregulatory role of T-cells. Phenotypic and functional analysis of T-cells in advanced periodontal disease reveal defective T-cell regulation, e.g. depressed CD4:CD8 ratios and depressed autologous mixed lymphocyte reaction in gingival tissues and peripheral blood of periodontitis patients when compared to those of healthy subjects (Taubman et al., 1984; Sosroseno and Herminajeng, 1995; Yamazaki et al., 1995; Mathur and Michalowiez, 1997). Little works have been concerned directly with the B-cells, although progressive periodontal lesion is a B-cell dominated lesion and hyperresponsiveness of B-cells have been cited as being important in the pathogenesis of periodontal disease (Bick et al., 1981; Carpenter et al., 1984; Ishikawa et al., 1997).

B-cells in periodontal disease

Tew et al. (1989) suggested that the polyclonal B-cell activation (PBA) contribute to hyperresponsiveness of B-cells in progressive periodontal disease. Several Gram negative and positive oral organisms possess the ability of PBA as shown by polyclonal antibody production and lymphocyte blastogenic response (Bick et al., 1981; Donaldson et al., 1982a). Such antibody production, which were previously thought to be protective, are now turned out to be non-protective since the major antibody response to the antigens e.g. lipopolysaccharide (LPS) was found to be weak at complement fixation and opsonization, hence resulting in ineffective antigen elimination (Polak et al., 1995). The study of Carpenter et al. (1984) demonstrated that in humans, PBA by periodontopathic bacteria required T-cells. This might be either physical cell to cell contact, or mediators release from T-cells or other mononuclear cells or both. Recently, it was shown that B-cells from other diseases such as systemic lupus erythematosus (SLE) could be induced to undergo spontaneous proliferation and differentiation into immunoglobulin producing cells in the absence of T-cells and monocytes (Tanaka et al., 1988). To date, there has been no further investigation into which mechanisms involving B-cell activation by periodontopathic bacteria since the study of Carpenter et al. (1984). Therefore, this area of research should be revisited. Because of major advances in instrumentation and methodology such as the fluorescence activated cell sorter, nucleic acid techniques and monoclonal antibodies, more sophisticated studies can now be conducted. Apart from lymphocyte proliferation assay and antibody production, we could monitor expression of surface activation antigens such as CD69, a very early activation marker, on B-cells or different immune cells after periodontopathic bacterial

stimulation. CD69 expression is the earliest inducible cell surface glycoprotein acquired during lymphoid activation both *in vitro* and *in vivo* under physiological conditions and inflammation. This molecule is involved in lymphocyte proliferation, and functions as a signal-transmitting receptor in T-cells, B-cells, NK cells and platelets (Santis et al., 1995).

Now it has become increasingly clear that cytokines such as interleukin-2 (IL-2), interleukin-4 (IL-4) and interleukin-6 (IL-6) involve in B-cell activation, proliferation and differentiation. Many of which were identified in gingival tissues from periodontitis patients (Fujihashi et al., 1991). Among these cytokines, high levels of IL-5 and IL-6 mRNA expression were present whereas IL-2 and IL-4 mRNA expression were not detected (Fujihashi et al., 1993a; 1993b). IL-10 was also found to be expressed on gingival mononuclear cells of the patients (Gemmell et al., 1995; Yamazaki et al., 1997). IL-10 plays a major role on suppressing immune and inflammatory response. It is produced by B-cells, T-cells and macrophages / monocytes (De Waal Malefyt et al., 1992). Human IL-10 reduces significantly the proliferation and production of cytokines by both Th1 (IL-2 and IFN-γ), and Th2 (IL-4, IL-5 and IL-6) clones exposed to specific antigen and phytohaemagglutinin (Del Prete et al., 1993). But it is a potent growth and differentiation factor for activated human B-cells (Rousset et al., 1992) and may therefore play an important role in amplifying B-cell response in the presence of periodontopathic bacteria in progressive periodontal lesion.

For many years, the only function known of B-cells has been antibody production, however it is now realized that a large number of inflammatory and regulatory cytokines are produced by activated B-cells. IL-1 and IL-6 are

important cytokines involving in periodontal tissue destruction and could possibly be produced by B-cells. Both cytokines appear to have a major role in the mediation of inflammation and immune responses initiated by infection and injury. IL-1 acts as a potent inducer of bone demineralization and also synergies with other cytokines such as tumor necrosis factor-α (TNF-α) and IL-6 in stimulating bone resorption (Dinarello, 1989; Gemmell and Seymour, 1993). IL-1 levels have been shown to be elevated in the gingiva of adult periodontitis subjects compared with clinically healthy or gingivitis affected individuals (Honig et al., 1989; Tokoro et al., 1996) and also in active periodontitis sites compared with inflamed stable sites (Stashenko et al., 1991). Furthermore IL-1 levels decrease after periodontal treatment (Masada et al., 1990; Matsuki et al., 1993). IL-6 has also been shown to be increased in to gingival crevicular fluid of patients with refractory periodontitis. High level of biologically active IL-6 was detected in gingival mononuclear cells isolated from inflamed gingiva (Fujihashi et al., 1993a; 1993b). Whether activated B-cells in response to periodontopathic bacteria such as *Porphyromonas gingivalis* contribute to IL-1 and IL-6 production, has not yet been demonstrated.

1.2 Objectives

1.2.1 To study the expression of CD69 on B-cells in *Porphyromonas gingivalis*-treated peripheral blood mononuclear cells (PBMC) and *P. gingivalis*-treated purified B-cell cultures.

1.2.2 To identify endogenously produced soluble mediators in *P. gingivalis*-treated PBMC cultures that stimulate B-cell activation.

1.2.3 To investigate the effect of *P. gingivalis* and IL-10 on induction of polyclonal B-cell activation in term of proliferation.

1.3 Hypothesis

Concurrent stimulation of B-cells by *P. gingivalis* together with soluble mediators released from activated mononuclear cells may represent the major mechanism underlying the polyclonal B-cell activation in progressive periodontitis.

1.4 Field of Research

To investigate the mechanism of polyclonal B-cell activation induced by Porphyromonas *gingivalis*.

1.5 Criteria Inclusion

1.5.1 Peripheral blood samples were collected from healthy adult volunteers.

1.5.2 Subjects who have healthy periodontium or gingivitis with probing pocket depth less than 4 mm. were included.

1.5.3 Subjects had not taken any antibiotics or anti-inflammatory drugs within 3 months prior to the beginning of the study.

1.5.4 *Porphyromonas gingivalis* FDC-381 were used.

1.5.5 Mononuclear cells were obtained from peripheral blood samples using density gradient centrifugation.

1.5.6 Purification of αβ T-cells, γδ T-cells, NK cells and B-cells were positively sorted from rosetting or non-rosetting population by using flow cytometry.

1.5.7 Cytokine production was analyzed by ELISA.

1.5.8 Cell surface marker analysis was determined by flow cytometry.

1.5.9 B-cell proliferation was analyzed by [^{3}H] thymidine incorporation.

1.6 Limitation of Research

This study cannot use many samples due to factors of time and expenses.

1.7 Application and Expectation of Research

Polyclonal B-cell activation is a hall mark feature of progressive periodontitis. The role of PBA in the pathogenesis of progressive periodontitis has remained undefined. It has been shown that the enhanced antibody production associated with PBA seems to be of little value to the host, since antigen-specific immunity does not develop. *P. gingivalis* has been reported to stimulate B-cells via lipopolysaccharide (LPS). The present study will determine which cytokines released in *P. gingivalis*-treated PBMC cultures, involved in B-cell activation. The combination effect of *P. gingivalis* and cytokines to promote B-cell proliferation and production of antibody and

cytokines will also be evaluated. Once finished, this study will provide a better understanding about the mechanism of PBA induced by *P. gingivalis*. Furthermore, it will contribute directly to the rational design of an immunological approach to suppress PBA in progressive periodontitis.