CHAPTER 2

Literature Review

2.1 Introduction

Periodontal disease is a chronic inflammation of the tooth supporting tissues caused by a complex interaction between the host defenses and microorganisms in dental plaque (Sosroseno and Herminajeng, 1995). Periodontal health can be seen as a balance between infection and immunity. If either side of the balance is altered, the balance is shifted away from health and towards disease (Zambon, 1994).

Periodontal disease could be separated into those that involve only the gingiva, which is called " gingivitis", and those that are associated with the destruction of the underlying structures of the periodontium, which is called " periodontitis " (Caton, 1989). Gingivitis is among the most common group of periodontal diseases. It is clinically characterized by increased redness, swelling and bleeding of gingiva upon brushing and gentle probing. Without any periodontal treatment, the gingival lesion may be confined to the marginal tissues and remain relatively stable which does not endanger the life of the dentition (Hirschfeld and Wasserman, 1978; McFall, 1982). On the other hand, periodontitis, the advanced form, causes gingival inflammation, loss of connective tissue, resorption of alveolar bone, and formation of periodontal pockets. The destruction process of advanced lesion is believed to be episodic with exacerbation and remission, but not continuous (Goodson et al.,

1982). More severe stages of the disease process may lead to a loosening and finally loss of teeth (William, 1990).

2.2 *Microbial Aspects of Periodontal Disease*

2.2.1 *Periodontopathic Bacteria*

It has been well recognized that periodontal disease is an infectious disease, which is caused by bacterial deposits along surfaces 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 now known that gingival inflammation often related to the amount of dental plaque but not to the specific pathogens. In contrast, at periodontitis sites, the specific groups of Gram negative anaerobes have been found to be the majority in subgingival plaque microorganisms. These include *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans* (Aa), *Bacteroides forsythus*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Eikenella corrodens* and *Treponema* species (Slots, 1977b; Dzink et al., 1988; Simonson et al., 1988; Genco et al., 1996).

2.2.2 *Porphyromonas gingivalis*

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. *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).

P. gingivalis possesses many virulent properties which enable itself to successfully colonize and compete in an ecological niche as well as the ability to evade the host. Examples of its virulent traits include the expression of special fimbriae which are implicated in adherence to gingival tissues (Yoshimura et al., 1984), secretion of polysaccharide capsules which prevent complement proteins and antibody from binding to outer surface (Schifferle et al., 1993a; 1993b) or wide varieties of enzymes which are considered as major virulent factors. Among these, collagenase and trypsin-like proteases which are very specific to *P. gingivalis*, are capable of degrading host immunoglobulin and complement. It was demonstrated that certain virulent strains of *P. gingivalis* could cleave the Fc regions of the bacterial-bound IgG, preventing phagocytosis and killing (Sundqvist et al., 1984; Schenkein, 1988). Thus, *P. gingivalis* interferes in the process of opsonization and is thereby able to evade neutrophil clearance. Interestingly, *P. gingivalis* has been shown to be able to invade human gingival epithelial cells *in vitro* (Duncan et al., 1993; Sandros et al., 1994). 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.

P. gingivalis does not have the lipopolysaccharide (LPS) typical of other Gram negative bacteria (Ishikawa et al., 1997). Although, the LPS of *P. gingivalis* shows very little endotoxic activity in classical endotoxin assays,

its carbohydrate complex appear to be highly antigenic and immunogenic (Mayrand and Holt, 1988; Ranney et al., 1991; Whitney et al., 1992). These carbohydrate antigens elicit a strong IgG2 isotype antibody response. This particular isotype of IgG is generally not regarded to be particularly effective in fixing complement or enhancing opsonization. High level of IgG2 antibody against *P. gingivalis* LPS was detected in adult periodontitis patients (Lopatin and Blackburn, 1992), therefore the humoral response to *P. gingivalis* may be ineffective in clearing this organism (Whitney et al., 1992).

2.3 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. On the contrary, the gingivally confined lesion or gingivitis, is characterized by a predominance of T-cells (Seymour and Greenspan, 1979; Seymour et al., 1979b; Matsuo et al., 1996). The factors causing the conversion from gingivitis to periodontitis have yet to be clarified. Seymour (1991) hypothesized that regulation of periodontal disease progression is under T-cell control which exists in a relatively stable lesion. But if loss of T-cell control occurs, a stable T-cell lesion would change to a progressive B-cell lesion.

Recently, 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 work has 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).

2.4 *Role of B-cells in Periodontal Disease*

2.4.1 *Introduction*

It is well recognized the role of B-cells in humoral immunity with its primary function being antibody production. Each B-cell is genetically programmed to encode a surface receptor, Ig, specific for a particular antigen. Having recognized its specific antigen, the B-cells multiply and differentiate into plasma cells, which produce large amounts of antibodies. These antibodies facilitate the removal and destruction of pathogenic agents by activating the complement cascade and by serving as tags for endocytosis, phagocytosis and antibody-dependent cellular cytotoxicity by macrophages, neutrophils, natural killer cells or eosinophils (Gold and Defranco, 1994).

2.4.2 T-dependent and T-independent Antigens

There are two types of antigens which induce antibody response i.e. T-dependent and T-independent. The activation of B-cells by T-dependent antigens requires T-B cell interaction or T-cell help. The interaction between T-cells and B-cells is a two way process in which B-cells present antigen to T-cells and receive signals from T-cells for division and differentiation. The central, specific interaction is that between major histocompatibility complex (MHC) class II-antigen complex on B-cells and the T-cell receptor, it also involves the co-stimulatory molecules e.g. B7-1 and B7-2 molecules on B-cells interact with CD28 on T-cells, which causes stabilization of mRNA of IL-2 and other cytokines in the T-cells, thereby prolonging the delivery of the activation signals. It is now recognized that CD40 delivers the most potent activating signal to B-cells, more potent even than signals transmitted via surface Ig. Upon activation, T-cells transiently express a ligand that interacts with CD40, termed CD40L. This interaction helps to drive B-cells into cell cycle (Tew et al., 1989; Noelle et al., 1992; Gold and Defranco, 1994; Pistoia, 1997).

During T-B interaction, T-cell can secrete a number of cytokines that have a powerful effect on B-cells. These include IL-2, a proliferation inducer for B-cells and T-cells, IL-4 which acts early in B-cell activation and proliferation, IL-5 which in the mouse (but not humans) is a powerful B-cell activator, and IL-6, which is a strong signal for B-cell differentiation. T-cells can also produce TNF-α and TNF-β. These molecules have been reported to be important for B-cell growth. Other cytokines which activate B-cells include IL-10, IL-12, IL-13 and IL-15. IL-12 acts in synergy with IL-2 on growth and differentiation of activated B-cells by enhancing B-cell proliferative response

and antibody production (Li et al., 1996). IL-13 mimics the actions of IL-4. This cytokine induces the expression of CD23 on purified B-cells together with the up-regulation of MHC class II antigens, and also induces isotype switching to IgG4 and IgE synthesis in immature human B-cells (Punnonen and de Vries, 1994). IL-15 resembles IL-2. It enhances B-cell expansion and antibody production as well as T-cell and NK cell proliferation and activation (Doherty et al., 1996). B-cells themselves can also secrete cytokines such as IL-1 and IL-6 which enhance expression of IL-2 receptor on the T-cells (Noelle et al., 1992; Gold and Defranco, 1994; Gemmell et al., 1997; Page et al., 1997).

The other type of B-cell antigens, T-independent, which are capable of activating B-cells without T-cell help have a number of common properties. In particular, they are all large polymeric molecules with repeating antigenic determinants. Many of them possess the ability, in high concentrations, to activate B-cell clones that are specific for other antigens, a phenomenon known as polyclonal B-cell activation (PBA). However, in lower concentrations they only activate B-cells specific for themselves. Many T-independent antigens are particularly resistant to degradation and most are of microbial origin, for example bacterial carbohydrates such as dextran and levan, and bacterial proteins such as flagellin and LPS, a major component of the outer membrane of Gram negative bacteria. Approximately one-third of all B-cells can be activated by T-independent pathway (Andersson et al., 1977; Tew et al., 1989). Until now, the mechanism by which T-independent antigens trigger B-cells without requiring T-helper (Th) cells is not fully understood. This type of B-cell response is short-lived and lack of IgG. This may be due to lack of co-stimulation via CD40L and lack of IL-2, IL-4 and IL-5, which T-cells produce in response to T-dependent antigens. T-independent antigens often

chiefly activate a subset of B-cells (B-1) expressing CD5. CD5+ B-cells are physically and functionally distinct from conventional B-cells. They tend to produce polyreactive antibodies, e.g. those reacting with exogenous antigens such as LPS (Casali and Notkins, 1989) and self antigens, including those recognized by autoantibodies involved in systemic autoimmunity single-strand DNA (Casali et al., 1987), Fc fragment of IgG (Hardy et al., 1987), or erythrocytes (Hayakawa et al., 1984) as well as in organ-specific autoimmunity insulin (Burastero et al., 1988). The absolute number of CD5+ B-cells often increases in association with some autoimmune diseases, such as rheumatoid arthritis (Hardy et al., 1987), and Sjogren's syndrome (Dauphinee et al., 1988).

2.4.3 Polyclonal B-cell Activation in Periodontal Disease

As previously mentioned, advanced lesion of periodontitis is characterized by dominance of B-cells and plasma cells, and polyclonal B-cell activation is believed to be a major significance in the development of B-cell lesions.

Daly et al. (1983b) demonstrated that lymphocytes from chronically inflamed tissue secreted Ig progressively throughout a seven day culture period and that addition of pokeweed mitogen had no stimulatory effect on this Ig production. These findings suggested that gingival B-cells were stimulated in *vivo* and were secreting Ig at the time of recovery from tissues. In support of these studies, Seymour et al. (1985) showed that the majority of the cells from adult periodontitis lesions was mature B-cells. By using flow cytometric analysis, they further demonstrated that tissue B-cells expressed a range of activation markers including CD23 and/or CD25, the former being expressed on early activated cells and the later representing a late activation marker

(Gemmell and Seymour, 1991). Significant numbers of those that expressed these markers were found in periodontitis lesions but low number in gingivitis. Among different Ig isotypes, IgG appears to be the major Ig class secreted by gingival cells (Fujihashi et al., 1993b). Also IgA and IgM secreting gingival cells can be found in low number. Taken together, Gemmell and Seymour (1991), therefore, hypothesized that these are memory B-cells which might be primed by various antigens and then migrate into periodontal lesions where they become activated polyclonally and differentiate into IgG secreting cells. Several Gram negative and Gram positive oral microorganisms possess the ability of PBA as shown by polyclonal antibody production and lymphocytes blastogenic response (Bick et al., 1981; Donaldson et al., 1982a; 1982b). Although, LPS on many Gram negative bacteria is well known as non-specifically activating B-cells or being a T-independent antigens (Casali et al., 1987). Carpenter et al. (1984) demonstrated that PBA induced by periodontopathic bacteria required T-cell help.

PBA may result in autoreactive antibody production which could be considered in the pathogenesis of periodontitis. Previous evidences have shown the presence of circulating antibodies to Type I collagen (Ftis et al., 1986) as well as increased proliferation of peripheral blood lymphocytes in response to Type I collagen (Mammo et al., 1982). More direct evidence for local production of these antibodies at sites of tissue injury came from increased numbers of gingival cells of periodontitis patients secreting autoantibodies to Type I and Type III collagens, as well as cells secreting rheumatoid factor (Hirsch et al., 1988a; 1988b). In contrast, these antibodies were rarely detected in peripheral blood of patients with adult periodontitis and only low level was present in the patients' serum.

CD5+ B-cells are thought to responsible for enhancing autoantibody production. They were found in higher number in periodontitis lesions than in peripheral blood obtained from the same patients and healthy subjects (Afar et al., 1992; Sugawara et al., 1992). Of importance, these autoreactive B-cells appeared to be in activated stage (Aramaki et al., in press) and may, therefore, play a non-protective role in periodontal disease.

2.5 *Cytokine Involvement in Pathogenesis of Periodontal Disease*

2.5.1 Introduction

Cytokines can be defined as small proteins (8-80 kDa molecular weight) that usually act in autocrine or paracrine manner. They are cell regulators that have a major influence on the production and activation of different effector cells. T-cells and macrophages are major source although they are produced by a wide range of cells that play important roles on physiological and inflammatory responses. The production of these potent molecules is usually transient and tightly regulated. Well over 100 different human cytokines have been identified (Rook and Balkwill, 1998). Cytokines generally act at picomolar concentrations and interact with specific receptors at the cell membrane, setting off a cascade that leads to induction, enhancement or inhibition of a number of cytokine-regulated genes in the nucleus (O'Garra et at., 1989a; 1989b).

Cytokines have a wide variety of names e.g. interleukin (IL), interferons (IFN), colony stimulating factors (CSF), tumour necrosis factor (TNF), growth factors and chemokines. They can have multiple functions and several cytokines may have similar actions. They are rarely produced, and rarely act

alone. Cytokines function in a complex network in which production of one cytokine will influence production of, or response to, several others. A cell will rarely be exposed to a single cytokine *in vivo*. The response of a cell to a given cytokine depends on the local concentration, the cell type, and other regulators to which it is constantly exposed (Balkwill and Burke, 1989; Cohen and Cohen, 1996).

2.5.2. Cytokines that Mediate the Immunoinflammatory Response on Periodontal Disease

Cytokines are recognized as being vital in the immunopathology of periodontal disease (Gemmell et al., 1997). They are produced in periodontium by infiltrating leukocytes and activated resident gingival cells such as resident fibroblasts, junctional epithelial cells, vascular endothelium, mast cells, and bone cells (Kornman et al., 1997). Cytokines comprise the major regulators of the immunoinflammatory responses that characterize periodontitis and are the major determinants of the outcome whether being protective or destructive (Kelso, 1990). Just how the immune system selects the particular response to a particular pathogen remains unclear.

Among different cytokines being involved in inflammatory process of periodontal disease, IL-1 appears to be a principal mediator in periodontitis (Page, 1991). IL-1β comes mostly from activated gingival macrophages and fibroblasts, and IL-1α produces from keratinocytes of the junctional or pocket epithelium (Kornman et al., 1997). Production of IL-1 is induced by LPS, other bacterial components and by IL-1 itself. IL-1 is one of the proinflammatory cytokines which include TNF-α, IL-6 and IL-8. All of these cytokines involve in the early inflammatory process of periodontal disease. They upregulate

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adhesion molecules on endothelial cells as well as leukocytes, therefore increase attachment between these cells and help to recruit immune cells into sites of inflammation (Bevilacqua et al., 1985; Kornman et al.,1997). These proinflammatory cytokines and their receptors have been identified in inflamed gingival tissues as well as gingival crevicular fluid (GCF) of the periodontal disease subjects (Kornman et al., 1997).

The other important role of IL-1 is bone destruction. IL-1 is the most potent known inducer of bone demineralization. It synergies with TNF in stimulating bone resorption (Stashenko et al., 1987) as well as major changes in connective tissue matrix (Qwarnstrom et al., 1989). Like IL-1, IL-6 appears to be a potent stimulator of osteoclast differentiation and bone resorption (Roodman, 1992) as well as inhibitor of bone formation (Hughes and Howells, 1993).

Numerous studies have consistently demonstrated high level of these destructive cytokines, in particular IL-1 and IL-6, in inflamed gingival tissues and GCF from the sites with severe periodontal breakdown (Honig et al., 1989; Reinhart et al., 1993; Takahashi et al., 1994). Furthermore, these cytokine levels decrease after periodontal treatment (Masada et al., 1990; Matsuki et al., 1993). TNF-α was also detected in GCF from periodontitis patients but the level was not as high as the IL-1β (Yavuzyilmaz et al., 1995).

Apart from those proinflammatory cytokines and other cytokines which involve tissue destruction, B-cell regulatory cytokines are also of importance in the pathogenesis of periodontal disease since B-cells and plasma cells are the features of advanced periodontal lesions. These cytokines include IL-2,

IL-4, IL-5, IL-6, and IL-10. Previous studies by Fujihashi et al. (1993a) demonstrated high levels of IL-5 and IL-6 mRNA expression in gingival mononuclear cells isolated from severe periodontitis patients but IL-2 and IL-4 mRNA expression were not detected. With the cytokine IL-4, conflicts still exist whether being detectable or undetectable in inflamed gingival tissues. IL-4 is required for the clonal expansion of antigen specific B-cells. Most studies failed to identify IL-4 mRNA or its protein in periodontitis lesion (Takeichi et al., 1994; Ebersole and Taubman, 1994, Fujihashi et al., 1996; Prabhu et al., 1996), whereas Yamazaki et al. (1997) and Manhart et al. (1994) notified the presence of IL-4 in the tissues.

Recently, a cytokine IL-10 has been consistently reported in periodontitis lesion either by the measurement of mRNA expression or protein concentration (Gemmell et al., 1995; Yamazaki et al., 1997; Salvi et al., 1998, Aramaki et al., in press). IL-10 plays a major role on suppressing immune and inflammatory response. It is produced by B-cells, T-cells and macrophages (De Waal Malefyt et al., 1992). It is also a potent growth and differentiation factor for activated human B-cells (Rousset et al., 1992) and plays a role in diminishing delayed type hypersensitivity reactions and other cell-mediated responses. Therefore, IL-10 may play an important role in amplifying B-cell response upon periodontopathic bacterial stimulation in advanced periodontal lesion and may be significant in periodontitis.