CHAPTER II

LITERATURE REVIEW

2.1 *Introduction*

Periodontal disease is chronic inflammation of tooth supporting structures including gingiva, connective tissue and alveolar bone (Williams, 1990). It is caused by complex interaction between host defenses and microorganisms in dental plaque (Seymour, 1991). Peridontal disease could be clinically defined into two distinct groups, gingivitis and periodontitis (Caton, 1989). Gingivitis appears as a gingivally confined inflammatory lesion normally shown as bluish red or red, edematous gingiva and bleeding upon probing. Without treatment, the lesion may remain confined to the gingival tissues and over lifetime of the individual, with no loss of alveolar bone and periodontal tissue attachment. This lesion, referred to as the " stable lesion " (Seymour et al., 1979a) and the patient called " well maintained " (Hirschfeld and Wasserman, 1978). On the other hand, periodontitis, the lesion is characterized by periodic or cyclical patterns of periodontal degradation (Goodson et al., 1982) consisting of periods of progressive interspersed with periods of stability. This type of lesion has been termed the " progressive lesion " (Seymour et al., 1979b).

2.2 *Etiology: Plaque microorganisms*

It has long been recognized that microorganisms in dental plaque and their products are the etiologic agents of periodontal disease (Socransky and Haffajee, 1992). The characteristic of bacteria in dental plaque from

the healthy or gingivitis sites and periodontitis sites are different. Even though, in human, there are 300-400 species of bacteria present in subgingival plaque, only 10-20 species would play an important role in pathogenesis of periodontal disease (Socransky and Haffajee, 1991). The majority (approximately 85%) of bacterial plaque associated with stable lesion are gram-positive aerobic or facultative anaerobic cocci such as *Streptococcus sanguis* and *S. mitis* (Slots, 1977a). On the other hand, the majority (approximately 80%) of plaque microorganisms found in peridontal pocket or associated with progressive lesion are gram-negative obligate anaerobes and the minority (approximately 20%) are gram-positive (Slots, 1977b). Gram negative anaerobes such as *Porphyromonas gingivalis*, *Bacteroides forsythus*, and *Actinobacillus actinomycetemcomitans* are known as key pathogens which cause human periodontitis. (American Academy of Periodontology, 1996)

2.2.1 Porphyromonas gingivalis

P. gingivalis, a Gram negative, anaerobic, non motile, asaccharolytic rod, is one of the most prominent periodontopathic bacteria. It is mostly present in subgingival plaque associated with the periodontitis lesions with rapid alveolar bone loss or deep periodontal pocket (Albander et al., 1990) and study of Napawongdee (1995) showed that 86.67% of severe periodontitis patients had *P. gingivalis* in subgingival plaque, but none was detected in the healthy subjects. Moreover this bacterium can also be found in recurrent periodontitis lesion (Choi et al., 1990) and rapidly progressive lesion (Slots, 1982; Slots and Dahlen, 1985)

This species possesses unique surface components and produces a wide range of virulence factors such as fimbriae, hemagglutinin, proteases, lipopolysaccharide (LPS), outer membrane protein, fatty acids, NH_3, H_2S, etc. (Macdonald et al., 1956; 1963; Ishikawa et al., 1997). Identical to Gram negative prokaryotes, *P. gingivalis* synthesizes LPS. However, the LPS from this oral pathogen is chemically different from the other classical LPS. The *P. gingivalis* LPS shows very little endotoxic acitivity in classical endotoxin assays (i.e. Limulus lysate assay or Schwartzman test), although it is significantly mitogenic (Mayrand and Holt, 1988). In tissue culture studies, the *P. gingivalis* LPS does function as a significant cytotoxin, as well as inducer of several host derived cytokines and chemokines (Holt et al., 1999).

While *P. gingivalis* produces a large number of hydrolytic, lipolytic and proteolytic enzymes, the proteins known as "proteinases" have received considerable attention. *P. gingivalis* proteinases have been found to exhibit significant *in vitro* activity against host proteins such as immunoglobulins (Killian et al., 1983), iron-transport and iron-sequestering proteins, matrix proteins, bactericidal proteins and peptides, and the proteins involved in coagulation, complement activity and kallikrein and kinin activity.

Immune response to the organism can be recognized by the high levels of specific IgG and IgA antibodies to *P. gingivalis* in serum (Naito et al., 1985; Ismaiel et al., 1988; Murayama et al., 1988) as well as in gingival

crevicular fluid (GCF) (Suzuki et al., 1984; Ebersole et al., 1985) of patients with adult periodontitis and rapidly progressive periodontitis. The antibody titre against this organism is often found to be higher in patients with periodontal disease than in healthy control subjects (Mouton et al., 1981). *P. gingivalis* was also shown to activate human peripheral blood monocytes *in vitro*. In the presence of the organism, monocytes produced significant amounts of Interleukin-1 (IL-1) and Tumor necrosis factor (TNF) (Linderman and Economou, 1988), pro-inflammatory cytokines which are important mediators involving tissue destruction.

Invasion is considered another important function of *P. gingivalis* since this mechanism affords protection from the host immune system and contributes to host cell as well as tissue damage. *P. gingivalis* has been shown to invade primary cultures of gingival epithelial cells (Lamont et al., 1995) as well as endothelial cells (Deshpande et al., 1998) and subsequently replicate intracellularly *in vitro*. The invasion results in the spread of the bacteria, which are well-equipped with virulence factors acting on deeper periodontal tissues of host. Even though, the invaded bacteria may eventually be destroyed by defense mechanisms such as antibody-dependent cytotoxic action via complement mediation, the bacterial components such as DNA fragment may consequently expose to surrounding connective tissues and activate local gingival cells. Recent studies (Cowdery et al., 1996; Sparwasser et al., 1997a,b) have shown that Gram negative and Gram positive bacterial DNAs are potent stimulators of several inflammatory cytokines both *in vivo* and *in vitro*.

Interestingly, *P. gingivalis* DNA could stimulate the expression of inflammatory cytokines e.g. IL-6 by human gingival fibroblasts (Takeshita et al., 1999), thus suggesting that this bacterial DNA may function as a virulence factor in periodontal disease.

2.3 *Pathogenesis: host response to plaque microoganisms*

Although microbial plaque is known to be essential to initiate disease and fuel progression, it is insufficient to explain the prevalence and severity of periodontitis. Host factors such as host defense mechanisms to dental plaque and environmental factors such as smoking are equally as important as determinants of disease occurrence and severity outcome (Page et al., 1997).

Histological studies reveal that healthy periodontium consists of very few inflammatory infiltrates including lymphocytes, macrophages and other cell types such as NK cells. As periodontal tissues become inflamed, more cellular infiltrates are evident. T cells are found to be predominant cells in the stable lesion or gingivitis whereas the proportion of B cells is increased in the progressive lesion or periodontitis (Seymour et al., 1979). The majority of T and B cells in periodontitis lesions appear to be in activated stage as monitored by CD69 expression , an early activation marker (Champaiboon et al., 2000). In addition to the characteristics of dense cellular infiltrates of activated T and B cells, high levels of cytokines, both inflammatory and regulatory including IL-1, TNF, IL-6 and IL-10 are also detected in periodontitis lesions (Gemmell et al., 1997). Such unique characteristics of human periodontitis, thus indicate immune dysfunction

which may possibly associate with the presence of periodontopathic bacteria in subgingival plaque .

It is well recognized that T cells not only play a major role in protection against infection but also in induction of the damage associated with the infection (Romagnani, 1993). Significant numbers of acitvated T cells are found in progressive periodontal lesions, many of which co-express HLA-DR (Okada et al.,1988; Reinhardt et al.,1988), CD45RO antigens (Gemmell , 1992) and CD69. T cells are essential for activation of B cells and other immunocompetent cells found in progressive lesions. Previous studies have suggested that a local T cell immunoregulatory imbalance may exist in human periodontal disease. Early phenotypic studies demonstrated a depressed CD4:CD8 ratio in cells extracted from adult periodontitis lesions when compared with peripheral blood and healthy tissue or tissue with gingivitis (Taubman et al., 1984; Seymour et al., 1987). Furthermore, these extracted gingival cells from periodontitis lesions appeared to have reduced ability to respond in an autologous mixed lymphocyte reactions (Cole et al., 1987; Seymour et al., 1986; Taubman, 1988).

2.3.1 *Th1 and Th2 cytokines*

Cytokines are central to the mechanism by which helper T cells regulate the immune response (Swain, 1991) and the production of appropriate cytokines is necessary for the development of protective immunity. The immune response to infection would appear to be regulated by the balance between Th1 and Th2 cytokines, these two pathways often being mutually exclusive, with one resulting in protection and the other in

progression of disease (Cox and Liew, 1992). On activation, antigen specific naïve T cells are believed to progress into Th0 cells which secrete a combination of Th1 and Th2 cytokines and then differentiate further into Th1 or Th2 cells with repeated antigen stimulation (Modlin and Nutman, 1993). Th1 cells differentially produce IL-2 and IFN-γ whereas Th2 cells secrete IL-4, IL-5 and IL-6 after activation by antigen or mitogen (Mosmann, 1991). The net effect of the Th1 cytokines is to enhance cell-mediated responses, while that of the Th2 cytokines is to suppress cell-mediated response and enhance humoral immune response (Modlin and Nutman, 1993).

The presence of IFN-γ appears to be a requisite for the induction of the Th1 subset. It is produced during an immune response by antigen specific T cells and natural killer cells recruited by IL-2 (O'Garra, 1989a). Its regulatory effects include the activation of macrophages to enhance their phagocytosis and tumor killing capability as well as activation and growth enhancement of cytolytic T cells and natural killer cells (O'Garra, 1989b). In addtion to IFN-γ, IL-12 also plays a key role in the differentiation of Th1 cells. It acts on both Th1 cells and their precursors, and part of this activity is due to the induction of IFN-γ production by T and NK cells. IFN-γ has a positive feedback effect by enhancing the production of IL-12 by monocytes and macrophage, whereas the cytokines IL-4 and IL-10 are powerful inhibitors of IL-12 production. While the differentiation into Th2 cells is dependent on the presence of IL-4, an important factor in the clonal expansion of antigen specific B cells (Scott, 1993), it has been suggested

that IL-12 may be the IL-4 equivalent for the differentiation of Th1 cells, and these two cytokines may determine the balance of Th1 and Th2 cells (Trinchieri, 1993).

Whether the Th1 and Th2 paradigm exits and perhaps may be a major determinant of the progression of periodontitis remains to be investigated. It has been studied by measuring the levels of various cytokines in gingival crevicular fluid and extracts of healthy and diseased gingival tissues, determining levels of cytokine messenger RNA in the tissue using immunocytochemical techniques or polymerase chain reaction on tissue extracts and by measuring cytokine production by mononuclear cells harvested from gingival tissue. Based on their work and published studies by others (Aoyagi et al., 1995), Gemmell et al. (1997) postulated that the Th1 response is associated with stable periodontal lesions, whereas a Th2 response leads to the production of nonprotective antibody and disease progression. Recently, more consistant findings of Th2 cytokines including IL-10 and IL-13 have been reported in periodontitis tissues, thus supporting the role of Th2 response in the pathogenesis of periodontal disease (Yamazaki et al., 1997; Aramaki et al., 1998). These findings are in agreement with the appearance of predominantly progressive B cell lesion. However, not all the data support the Th2 response, other investigators observed the mixture of Th1 and Th2 responses in periodontitis tissues (Fujihashi et al., 1996). The data regarding the Th1 and Th2 paradigm in periodontal disease appear to be inconclusive.

2.3.2 *Inflammatory cytokines*

Inflammatory cytokines released during the disease process of continuous/excessive local host responses to plaque bacteria also play a crucial role in the pathogenesis of periodontitis. It has been shown by many investigators that significant levels of inflammatory cytokines such as IL-1α, IL-1β, TNF-α, IL-6 and IL-8 can be detected in GCF and inflamed periodontal tissues (Charon et al., 1982; Seymour et al., 1986; Masada et al., 1990; Rossomando et al., 1990; Stashenko et al., 1991a,b; Geivelis et al., 1993; Matsuki et al., 1993; Payne et al., 1993; Lee et al.,1995).

IL-1 is a principal mediator of inflammatory responses acting on many cell types and is itself produced by many different cells including macrophages endothelial cells, B cells, fibroblasts, epithelial cells ,and osteoblasts in response to microorganisms, bacterial toxins, complement components or tissue injury (Dinarello, 1987). IL-1α and IL-1β share only 27% homology at the amino acid level, but they have similar biological functions. IL-1 levels have been shown to be elevated in GCF (Masada et al., 1990) at periodontitis sites and in gingiva of adult periodontitis subjects compared with clinically healthy or gingivitis individuals and marked reductions of total IL-1 levels were observed following effective treatment (Masada et al., 1990; Matsuki et al., 1992). The local excessive production of IL-1 by cells composing the periodontium appears to be capable of stimulating gingival and periodontal ligament fibroblasts to induce the production of other cytokines, matrix-degrading enzyme, and prostaglandin E_2 (PGE_2). These mediators may be responsible for effecting connective tissue destruction, leading to loss of attachment.

TNF-α also has been suggested to have similar effects as IL-1. It induces secretion of collagenase by fibroblasts and resorption of cartilage and bone (Elias et al., 1987; Meikle et al., 1989). Both TNF-α and IL-1 have been shown to act on endothelial cells to increase the attachment of polymorphonuclear leukocytes and monocytes and thus help to recruit these cells into sites of inflammation (Bevilacqua et al., 1985). In addition, IL-1 synergizes the bone resorptive actions of TNF-α (Bertolini et al., 1986; van der Pluijm et al., 1991). TNF-α is produced mainly by macrophages in response to agents such as LPS (Linderman et al., 1988).

Macrophages constitute from 5-30% of the infiltrating cells in inflamed periodontal lesions and were thought to play a central role in the production of IL-1 in inflamed sites (Oppenheim et al., 1986; Page,1991). Matsuki and co-workers (1992) demonstrated by in situ hybridization, that IL-1α and IL-1β mRNA-expressing cells in the inflamed gingival tissues were mostly macrophages. Furthermore, mRNA expression of TNF-α, IL-6 and IL-8 was also detected in the macrophages in inflamed periodontal tissue. The major function of IL-6 is the induction of the final maturation of B cells into immunoglobulin-secreting plasma cells and also regulation of bone turnover (Ishimi et al., 1990; Kurihara et al., 1990). IL-8 is a potent chemotactic factor for leukocytes (Bickel, 1993; Skerka et al., 1993), thus being important of cell recruitment into local periodontal lesion.

2.4 *WF10*

WF10 is the code name of an Immunokine®. It is a 1:10 dilution of tetrachlorodecaoxygen anion complex. TCDO is a chemically stable,

water-soluble compound containing oxygen in a chlorite matrix. TCDO, in conjunction with hemo-proteins, forms an activated TCDO-heme complex in the tissue (Youngman et al., 1986). This complex has been hypothesized to be responsible for macrophage activation. The complex is relatively active, unstable and is broken down into heme, oxygen, chloride and water. In human TCDO has been shown to improve oxygenation of poorly healed wounds, resulting in enhanced healing and regeneration (Hinz et al., 1986). The double blind randomized clinical trial on 271 inpatients with difficult wounds using TCDO and 0.9% saline as control provided good evidence for wound healing properties of TCDO (Hinz et al., 1986). They showed that wound cleansing was intensified, the formation of new tissue (granulations, epithelium) was promoted, and irrespective of different wound types, wound surfaces decreased more quickly.

Importantly, WF10 has been claimed by the manufacturer for its immunomodulatory activity by influencing cell proliferation and function. This includes proliferation of immunocompetent cells, phagocytic index, oxidative burst, cell signaling, and immunoglobulin production. WF10 was shown to stimulate dose-related phagocytosis, humoral immune response, and cellular defense systems by modifying the function of the monocyte/macrophage system, natural killer cells (NK cells), and cytotoxic T cells. Macrophages are thought to be major target cells for WF10. The key feature of WF10 is the ability to activate macrophages to enhance phagocytosis and wound healing. For example, TCDO-hemoglobin complex was shown to accelerate candida phagocytosis by peritoneal

macrophages in mice (Woerly et al., 1987). Moreover, supernatants of mononuclear leukocyte cultured in the presence of TCDO were demonstrated to enhance the chemiluminescent activity both of resting and peptide formyl-Met-Leu-Phe (FMLP)-stimulated PMNs (Schopf et al., 1995). In addition to its immunostimulatory effects, the recent study by McGrath et al. (1998) showed that WF10 inhibited T cell proliferative responses to a variety of antigens such as tetanus toxoid and key hole-limpet hemocyanin (KLH). This effect was suggested to be due to the reduced antigen presentation *in vitro.*

Another interesting activity of WF10 is anti-microbial effect. Stoll et al. (1993) demonstrated the ability of WF10 to inhibit growth of 276 different strains of bacteria derived from the oropharyngeal flora. Of interest, these bacteria included Gram negative periodontopathic bacteria such as *P. gingivalis*, *A. actinomycetemcomitans*, *F. nucleatum*, and *W. recta.* Based on these described features of WF10, such claimed properties may be of interest to use as a therapeutic agents in periodontitis.