CHAPTER 1

Introduction

1.1 Background of present study

Periodontitis is one form of a chronic inflammatory periodontal disease (CIPD) that affects approximately 60 % of adult population in Thailand (The Fourth National Oral Health Survey, Ministry of Public Health, 1994). The other form of CIPD is gingivitis which was previously thought to inevitably lead to the severe form, periodontitis. Current information, however, indicates that gingivitis apparently does not always lead to periodontitis (Lindhe, Hamp and Loe, 1973) and this would involve local bacterial etiologies and host-parasite interactions.

Gingivitis appears as a gingivally confined inflammatory lesion normally shown as bluish red / red, edematous gingiva and bleeding upon probing. Without treatment, the lesion may remain confined to the gingival tissues and over the lifetime of the individual, further loss of supporting structure such as alveolar bone and periodontal tissue attachment does not occur. This lesion, referred to as the "stable lesion" (Seymour et al., 1979) and the patient called "well maintained" (Hirschfeld and Wasserman, 1978) may represent the most common form of the disease, particularly in children or young adults.

Periodontitis is clinically characterized by the presence of gingival inflammation, pocketting, and loss of alveolar bone and probing attachment loss. The nature of periodontal breakdown is periodic or cyclical (Goodson et al., 1982). This type of lesion has been referred to as the "progressive lesion" and the patient called "down hill" (Hirschfeld and Wasserman, 1978).

Clearly, the immunological and microbiological studies have shown the differences between the stable lesion and the progressive lesion. Early immunohistological studies have suggested that the predominant lymphocyte in the infiltration of the stable lesion is the T-cell, while an increase in the numbers of B-cells and plasma cells can be demonstrated in the progressive lesion (Seymour et al., 1979). The characteristic of bacteria in dental plaque from the two lesions 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 CIPD (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) and gram-positive rod such as *Actinomyces viscosus*, *A. israelii* while the minority (approximately 15 - 25%) are gram-negative anaerobic rod such as *Bacteroides*, *Fusobacterium* and *Veillonella* species (Slots, 1977a; 1977b). On the other hand, the majority (approximately 80%) of plaque microorganisms found in periodontal pocket or associated with

progressive lesion are gram-negative obligate anaerobes and the minority (approximately 20%) are gram-positive (Slots, 1977b). The bacteria commonly found in the progressive lesions are *Porphyromonas* (formerly *Bacteroides*)(Shah and Collins, 1988) *gingivalis*, *Actinobacillus actinomycetemcomitans*, *Bacteroides forsythus*, *Fusobacterium nucleatum*, *Wollinella recta* and *Prevotella intermedia* (Moore et al., 1991; Dzink, Socransky and Haffajee, 1988).

Among the most extensively studied of the putative anaerobic bacterial pathogens is *Porphyromonas gingivalis*. This microorganism is mostly present in subgingival plaque associated with the periodontitis lesions with rapidly alveolar bone loss or deep periodontal pocket (Albander, Olsen and Gjermo, 1990). 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). The recent study at the Faculty of Dentistry, Chulalongkorn University revealed that 86.67% of severe periodontitis patients in the Oral Diagnosis Clinic had *P. gingivalis* in subgingival plaque, but none was detected in the healthy subjects (Napawongdee, 1995).

P. gingivalis possesses a pathological ability to periodontium. Its fimbriae appears to mediate adherence to oral epithelial cells and salivary pellicle-coated tooth surfaces. Isogai et al. (1988) used the monoclonal antibodies to *P. gingivalis* fimbriae to block adhesion of the organism to human buccal epithelial cells. Furthermore, Lee et al. (1992) demonstrated that *P. gingivalis*

could bind to saliva - coated hydroxyapatite beads in a dose-dependent manner and this binding was inhibited by purified fimbriae.

When the bacterium adheres, the vesicles containing lipopolysacharide (LPS), proteolytic enzymes such as collagenase, protease, phospholipase (Smalley et al., 1989; Socransky and Haffajee, 1991), and trypsin - like enzyme were released and entered gingival tissues, triggerring a host response leading to direct tissue destruction. Cell wall fragments of *P. gingivalis*, which contain LPS, could activate peripheral blood B-cells resulting in polyclonal B-cell response with a number of B-cells, plasma cells and antibodies (Tew, 1985). Most of the antibodies produced are nonspecific as shown by Tew et al.(1985) after B-cell stimulated with *P. gingivalis*. These workers measured the specific antibodies to sheep red blood cells. The amount of the antibodies to sheep red blood cells produced after *P. gingivalis* stimulation was at the same level as when stimulated by pokeweed mitogen (PWM), therefore implying that these nonspecific antibodies do not effectively protect the periodontium. Furthermore, in human peripheral blood mononuclear cell (PBMC) cultured with whole cell of *P. gingivalis*, the culture supernatant was found to contain high concentration of Interleukin-1 (IL-1) (formerly called osteoclast activating factor) which is an important factor responsible for inflammatory response and bone loss(Lindemann and Economour, 1988).

It is now generally agreed that polyclonally induced immunoglobulin (Ig) production in human is regulated by T-cells (Rosenkoetter et al., 1984). Carpenter et al. (1984) have demonstrated T-cell control of polyclonal activation of peripheral blood B-cells induced by periodontopathic bacteria. In this study, when T-cells were depleted from human PBMC and were cultured with sonic extracts of a panel of oral bacteria and PWM, they showed the level of IgG and IgM in the culture supernatant were decreased. And when T-cells were added to the culture, the level of both Ig was significantly increased.

More evidence for the role of T-cell control in periodontal lesion came from animal studies. Yoshie et al. (1985) studied in athymic nude rats and T-cell-reconstituted nude rats. They found that these congenitally athymic (nude rnu/rnu) rats showed periodontal destruction with bone loss. Following reconstitution, the animals displayed normal T-cell function and normal IgG levels. The nude non-reconstituted rats contained predominantly B-cells in their gingiva as compared with the reconstituted and normal animals, which the lesions were predominately T-cells. There was increased periodontal bone loss in the nude rats (B-cell lesions) as compared with that in the normal animals (T-cell lesions), while the reconstituted animals showed decreased bone loss (T-cell lesions).

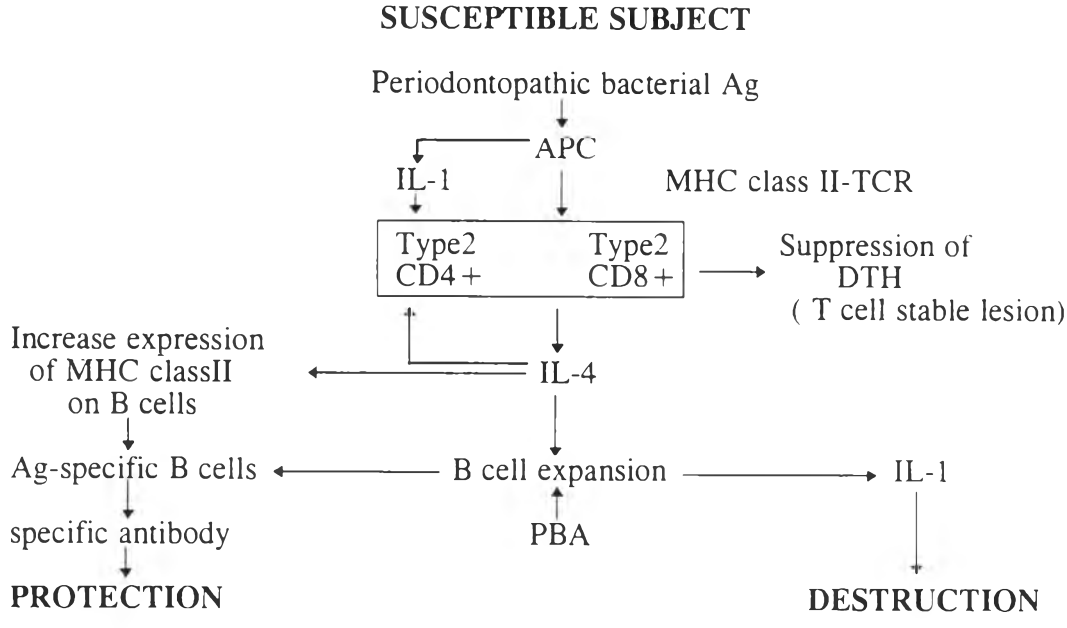
Hence, the results from both animal and human studies were taken to support the concept that progressive periodontal disease is primarily a B-cell lesion and that T-cells have a regulatory role in controlling this excessive B-cell response.

It was then postulated that the conversion for a stable lesion to a progressive lesion may be due to an imbalance of T-cell control in the stable lesion which may be induced by periodontopathic bacteria such as *P. gingivalis*.

Mahanonda (1992) established human peripheral blood T-cell lines and T-cell clones specific to *P. gingivalis* and *F. nucleatum* from healthy periodontal subjects. It was shown that the two bacteria could induce both types of T-helper (Th) cells, i.e. Th1 and Th2. In general, the Th1 and Th2 cells have been shown to have a functional dichotomy based essentially on their cytokine profiles. Th1 cells produce IL-2 and interferon (IFN)-γ, while Th2 cells produce IL-4, IL-5 and IL-6. The major function of the Th1 subset is to mediate delayed-type hypersensitivity reactions and their secondary function is suppression of B-cell activity. In contrast, the major function of the Th2 subset is to provide B-cell help, while their secondary function is cell mediated immune suppression (Street and Mosmann, 1991). A similar dichotomy has also been described for CD8+ T-cells (Bloom et al., 1992). The immune response to infection would appear to be regulated by the balance between Th1 and Th2 cytokines. Perhaps the presence of both Th1 and Th2 cells from healthy periodontal subjects in the study of Mahanonda (1992) suggests that the cytokines from both Th subsets could control the balance of immune response and this might occur in the stable lesion. Such a balance might not exist in the progressive lesion of the periodontitis patients. The factor involves may be the induction of the putative periodontopathic bacteria such as *P. gingivalis*.

Recently the roles of Th1 and Th2 cells in periodontal disease have been in focus. Seymour et al. (1993) have proposed the concept of both cell types in their hypothetical cellular and molecular model of CIPD (Figure 1). In this model, the non-susceptible subjects or the subjects with stable lesions may have Th1 cell types homing to the gingiva while the susceptible subjects or the periodontitis patients with progressive lesions may have Th2 cell types. Even though, it has become clear that patient susceptibility is of utmost importance to the outcome of the disease, the differences in host reactivity to the putative periodontopathic bacteria e.g. *P. gingivalis* may relate to the differrences in the regulation of the immune response.

To further investigate this concept, the cells from peripheral blood and gingival tissues were studied. The study of cells from the lesions have faced many problems, in particular a very low number of cells. Wassenaar et al. (1995) established a human gingival T-cell clone specific to *P. gingivalis*. In this study gingival cells were extracted from 4 periodontitis patients, however only one specific clone could be established. The investigation of this one clone showed the absence of IL-4 and IFN-γ production. However, it is difficult to draw a valid conclusion from this study. The limitation of the tissue culture experiment is due to the quantity of immunocompetent cells in periodontal lesions which is unabundant. Therefore, in the present study, peripheral blood T-cells from the severe periodontitis patients will be the source of cell culture and the T-cell lines reactive to *P. gingivalis* will be established.



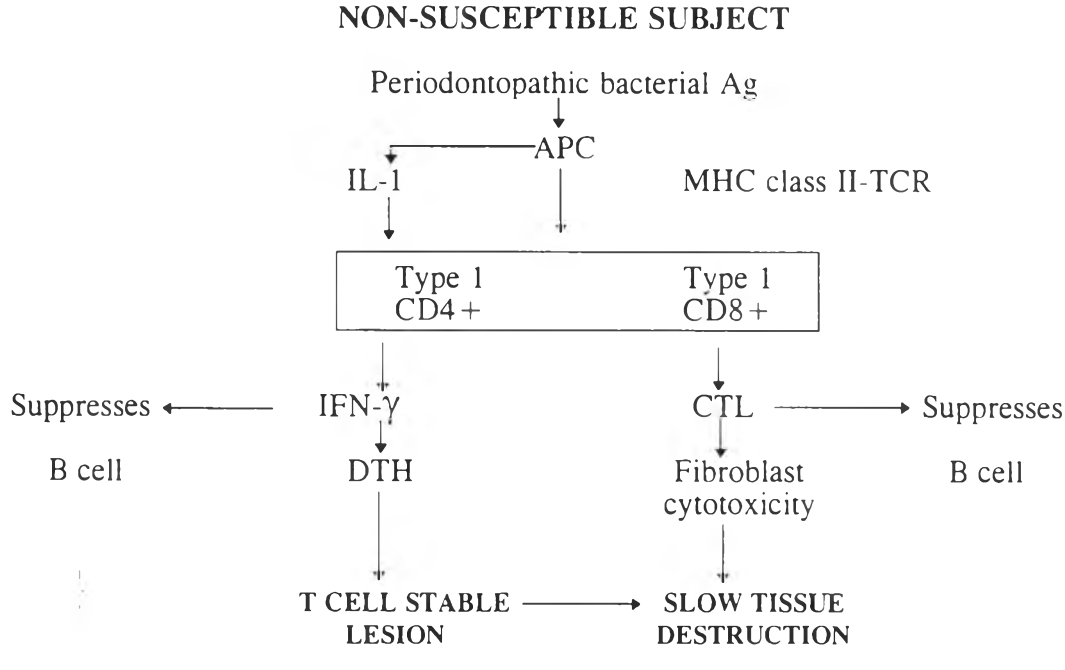


Figure 1: Hypothetical cellular and molecular model of chronic inflammatory periodontal disease

1.2 Objectives

1.2.1. To establish peripheral blood T-cell lines reactive to *P. gingivalis*

1.2.2. To investigate these antigen reactive T-cell lines in terms of

1.2.2.1 their specificity

1.2.2.2 their surface phenotypes

1.2.2.3 their cytokine profiles (IL-4 and IFN-γ)

1.3 Field of Research

Cell extracted from peripheral blood of severe periodontitis Thai patients age 35 - 65 years old.

1.4 Criteria Inclusions

1.4.1. Peripheral blood had been taken from 2 severe periodontitis patients who seek for treatment at Periodontal Clinic, Faculty of Dentistry, Chulalongkorn University.

1.4.2. The patients generally had good physical health except for having severe periodontitis.

1.4.3. The patients had not taken any antibiotics or steriod for at least 3 months

1.4.4. The severity of periodontal conditions of each patient would be as follows:

1.4.4.1 The patients were diagnosed as generalized adult periodontitis with a few hopeless teeth.

1.4.4.2 Such teeth had probing pocket depth deeper than 6 mm with periodontal attachment loss at least 5 mm.

1.4.4.3 The level of alveolar bone support was less than one third of root length. And these teeth were clinically mobile with third degree mobility.

1.4.4.4 The hopeless teeth were no periapical lesion.

1.4.5. Peripheral blood T-cell lines reactive to *P. gingivalis* would be established from each patient.

1.4.6. These antigen reactive T-cell lines would be investigated in terms of

1.4.6.1 Their specificity by proliferation assay

1.4.6.2 Their surface phenotypes by flow cytometry

1.4.6.3 Their cytokine production by enzyme immunoassay kit

1.5 Limitation of Research

According to time limitation and high expenses, peripheral blood T-cell lines from two severe periodontitis subjects will be established and investigated.

1.6 Application and Expectation of Research

This study will involve T-cell culture from peripheral blood of periodontitis patients. Briefly, T-cells will be extracted and stimulated with periodontopathic

bacteria in order to develop into specific T-cell lines. Hence, there will be enough number of antigen specific T-cells to investigate their characteristics and function and in particular a pattern of cytokine production.

It is now well recognised that cytokines are central to the mechanism by which T-cell regulate the immune response and the production of appropriate cytokines is necessary for the development of protective immunity. On the other hand the production of nonprotective or inappropriate cytokines could result in tissue damage. Understanding of cytokine profiles produced by T-cells specific to periodontopathic bacteria, *P. gingivalis* in periodontitis patient would link T-cell subsets to disease susceptibility and expression and would provide more depth into immunopathogenesis of chronic inflammatory periodontal disease. Subsequently, this could lead to the possibility of forming the basis of new immunological approaches for prophylaxis and treatment of the disease such as cytokine therapy which may be commonplace in the future. Obviously, the benifits would be for all.