CHAPTER 2

Literature Review

2.1 Introduction

Clinically, periodontal disease in adults has been shown to manifest itself as at least two distinct entities (Hirschfeld and Wasserman, 1978; Mcfall, 1982). One form is relatively stable and does not endanger the life of the dentition. Patients who manifest this form of the disease are not susceptible to periodontal breakdown and have been called "Non-susceptible subjects" (Seymour, et al., 1993). The lesion itself has been termed the "stable lesion" (Seymour et al., 1979b). The second form of the disease is characterized by periodic or cyclical patterns of periodontal degradation (Goodson et al., 1982) consisting of periods of progression interspersed with periods of stability. This type of lesion has been termed the "progressive lesion" (Seymour et al., 1979b). The patients who are susceptible to periodontal breakdown and exhibit these advanced lesions have been called "Susceptible subjects" (Seymour, et al., 1993).

2.2 Microbial aspects of CIPD

2.2.1 Plaque bacteria

A great deal of research during the past decades has indicated that microorganisms in dental plaque are the cause of periodontal disease. But, the true nature of the relationship between dental plaque and CIPD remains unclear. In as

much , no species of plaque microorganisms has been shown to fulfil Koch's postulates, which must mitigate against classical specific microbial pathogenicity (Socransky, 1991).

Current evidence indicates that plaque is a part of the natural human microflora. There are approximately 10^{10} bacteria per gram wet weight (Slots and Listgarten, 1988) or at least 300 bacterial species (Moore, 1987) harboring on the tooth surface and in the periodontal pocket. However, there seems to be an association between specific organisms in dental plaque and the different disease states.

According to direct microscopic examination of subgingival plaque found in healthy periodontal sulci, nonmotile rods and cocci constitute approximately 95% of the total microflora and spirochetes are only rarely encountered (Listgarten and Hellden, 1978). These microorganisms were identified as gram-positive facultative organisms (85%) usually of the genus *Streptococcus* and *Actinomyces* (Slots,1977a).

Microscopically, the development of gingivitis is accompanied by a decrease in the proportion of cocci and by a parallel increase in motile rods and spirochetes (Lindhe et al., 1980; Tempro et al., 1983). Gram-negative bacteria constitute approximately 45% of the total bacterial recovery (Slot et al., 1978). Besides *Actinomyces* species and *Streptococcus* species, *Fusobacterium* species,

and *Prevotella intermedia* (formerly *Bacteroides intermedius*) and various non-pigmenting *Bacteroides species* are also found in gingivitis plaque. Generally such plaque composition is found to be similar to plaque associated with periodontal health. The major difference is in the total number of organisms where in gingivitis, there is approximately ten to twenty fold increase (Van Palenstein Helderman, 1975; Loesche and Syed, 1978). This significant increasing in the number of bacteria appears to have a greater pathogenic effect on periodontal tissues in gingivitis than the relatively minor and inconsistent shifts in the microbial composition, therefore suggesting a nonspecific microbial etiology for gingivitis.

In marked contrast, unusual forms of gingivitis and advancing or destructive periodontal disease appear to be associated with a group of specific bacteria found in the subgingival microflora. Further support for the specific plaque hypothesis may be from the fact that plaque varies from person to person and from site to site within the same person (Bowden et al., 1975; MacFarlane et al., 1988).

In adult periodontitis, the proportions of motile rods (14%) and spirochetes (38%) are further increased (Listgarten and Hellden, 1978; Listgarten, 1984), the cultivable microflora comprises about 75% gram-negative and 90% anaerobic organisms (Slots, 1977b). Common gram-negative isolates include *Porphyromonas gingivalis* (previously designated as *Bacteroides gingivalis*, Shah

and Collins, 1988), *P. intermedia*, *Fusobacterium* species, non-pigmenting *Bacteroides* species, *Wollinella species* and *Actinobacillus actinomycetemcomitans* (Slots, 1977b; Tanner et al., 1979; Zambon et al., 1981; Savitt and Socransky, 1984).

In localized juvenile periodontitis, motile organisms are found prominent in plaque although they are not as numerous as in adult periodontitis (Liljenberg and Lindhe, 1980). The flora of this particular lesion consists mainly of gram-negative rods (65%) and anaerobic bacteria (80%) (Slots, 1976) which are *A. actinomycetemcomitans*, *P. intermedia*, *Capnocytophaga* species, and *Eikenella corrodens* (Tanner et al., 1979; Slots and Rosling, 1987; Zambon et al., 1983).

Although recent reviews have favored the concept of bacterial specificity in various forms of periodontal disease, it remains unclear whether the microbial composition at the lesion site reflects those organisms responsible for the observed pathology, or organisms which were resident as a result of a changed environment. However, the putative periodontopathic bacteria all exhibit marked pathogenic potential in the form of toxins and enzymes, which can lead to destruction of the gingival connective tissues and surrounding alveolar bones (Slots and Genco, 1984).

2.2.2 *Porphyromonas gingivalis*

It is now well recognized that there is an association between *P.gingivalis* and periodontal disease which is based on microbiological and serological studies in human and non-human primates (Slots and Listgarten, 1988; Van Winkelhoff et al., 1988). As mentioned earlier, *P. gingivalis*, a gram-negative, nonmotile, anaerobic short rod, is frequently found at the sites associated with adult periodontitis lesion but rarely found at the healthy sites (Van Winkelfhoff et al., 1988).

Additional evidence for the role of this organism in periodontal disease comes from studies on human antibodies reactive with the organism. Several laboratories have shown that adult periodontitis patients on average exhibit a higher serum antibody level to *P. gingivalis* than healthy periodontal subjects and children (Mouton et al., 1981; Altman et al., 1982; Doty et al., 1982; Taubman et al., 1982; Tolo and Brandtzaeg, 1982; Naito et al., 1984; Suzuki et al., 1984; Tolo and Schenck, 1985; Vincent et al., 1985; Ebersole et al., 1986; Martin et al., 1986). Isotype analysis of antibody to whole cells or to protein and carbohydrate antigens of *P.gingivalis* revealed that the major response was IgG followed by IgA and IgM (Ogawa et al., 1990). A local antibody response to *P. gingivalis* was also found in gingival crevicular fluid (Schonfeld and Kangan, 1982; Ebersole et al., 1984; Smith et al., 1985; Choi et at., 1990). From the relationship between the total IgG and albumin concentrations in serum and gingival crevicular fluid, it has been estimated that about half of the IgG in gingival crevicular fluid is being

produced by the local plasma cells (Tew et al., 1985). Moreover, it has been shown that high concentration of *P.gingivalis* specific IgG and IgA antibodies were present in gingival homogenates of adult periodontitis patients (Smith et al., 1985). These antibodies were previously thought to be protective, in the severe stage of periodontal disease this might not be the case. Fujihashi et al. (1993) demonstrated the elevated frequency of IgG2 and IgG4 subclass antibody-secreting cells in inflamed tissues of the advanced stage of periodontal disease when compared with moderate stages. These two subclasses of IgG molecules have been shown to be ineffective antibodies for fixing complement and for enhancing effector cells. Furthermore, increased appearance of IgA2 subclass plasma cells was also detected. Perhaps, the production of such anti-inflammatory antibodies(e.g. IgA2, IgG2 and IgG4) in the advanced stage of disease might have been too late to overcome the presence of periodontopathic bacteria or the severe inflammation itself.

Pathogenic strategies of P. gingivalis

2.2.2.1 Ability to adhere and colonize

The ability of *P. gingivalis* to adhere to subgingival, buccal and crevicular epithelial cells, to tooth surface as well as to the surface of other bacteria, is thus a crucial step in its successful establishment within the oral cavity. The products of *P. gingivalis* including fimbriae, hemagglutinating factors, proteases, extracellular membrane vesicles, LPS and a polysaccharide (PS) capsules

all appear to contribute to the interaction with and attachment of *P. gingivalis* to these subgingival tissues (Cutler et al., 1995).

P. gingivalis fimbriae have been shown to mediate adherence to oral epithelial cells, to salivary-pellicle-coated tooth surfaces, and to other bacterial species e.g. *Actinomyces viscosus* and *Streptococcus sanguis* (Genco et al., 1994). Fimbrillin, a subunit of fimbriae appears to be responsible for binding to surface-bound salivary components. Isogai et al.(1988) showed that monoclonal antibodies to *P. gingivalis* fimbriae blocked adhesion of *P. gingivalis* to human buccal epithelial cells. Lee et al. (1992) showed that *P. gingivalis* could bind to saliva-coated hydroxyapatite beads in a concentration-dependent manner and this binding was inhibited by purified fimbriae. Fimbriae purified from various *P. gingivalis* strains differ in size, amino-terminal sequences and antigenicity (Genco et al., 1994). Immunization with purified naive fimbriae has been shown to protect against periodontal tissue destruction in the gnotobiotic rat model (Evans et al., 1992). Therefore, *P. gingivalis* fimbriae are important in virulence, possibly by mediating adherence and colonization of the oral cavity, which may be the first step in the initiation of periodontal destruction.

After *P. gingivalis* has adhered to host tissues, it must utilise essential nutrients to colonize and grow. *P. gingivalis* will grow in the presence of iron from hemin (Genco et al., 1994). The characteristic black pigmentation of its colonies appears to be due to the accumulation of hemin on the cell surface. A clinical

symptom of periodontitis, bleeding periodontal pockets, indicate good source of erythrocytes or hemin for the organism. *P. gingivalis* can bind to the erythrocytes via hemagglutinins such as HA-Ag2 (Mouton and Chandad, 1993) and lyse them by hemolysin, thus liberating hemin (Chu et al., 1991). Several investigators have shown that hemin influences the virulence of *P. gingivalis*. Growth of the organism when hemin is limited increase the number of extracellular vesicles, the level of the hemolytic trypsin-like protease and hemagglutinating activity (Cutler et al., 1995).

P. gingivalis also requires small peptides and amino acids for growth which can obtain by degrading host tissue proteins such as collagen, elastin, fibrinogens and serum proteins. This is believed that potent proteases produced by *P. gingivalis* serve this purpose (Lantz et al., 1991).

2.2.2.2 Immune-evasion of *P. gingivalis*

Current evidence indicates that *P. gingivalis* resists phagocytosis by polymorphonuclear(PMN) by producing a unique PS and proteases that degrade complement component C3 and IgG (Sundqvist et al., 1991; Cutler et al., 1993). These factors appear to prevent serum opsonization, serum killing and adherence to various host cells e.g. PMN. PS may inhibit the activation of complement by masking the complement-activation LPS on the surface (Schifferle et al., 1993). Thus, the production of PS, C3 and IgG degrading proteases appear to protect *P. gingivalis* from the effector functions of serum component and PMN.

P. gingivalis also has a direct effect on PMN. It produces heat-stable nonproteolytic factors with porin-like activity that can depolarize PMN membranes and immobilize PMN responses to chemotactic factors (Novak and Cohen, 1991). Furthermore, *P. gingivalis* has been shown to inhibit the phagocytic activity of PMN directly, by altering the expression of PMN Fc receptors for IgG, CR1 and CR2 (Tai et al., 1993).

2.2.2.3 Tissue destruction

P. gingivalis has been observed within gingival tissues, as well as colonizing mucosal surfaces; it may also pass through epithelial barrier. Duncan et al. (1993) have reported that certain strains of *P. gingivalis* can bind and invade the human oral epithelial cell line. This ability to invade host tissues might result in direct host tissue destruction and/or host-induced inflammatory response. *P. gingivalis* elaborates potentially cytotoxic products such as indole, ammonia, hydrogen sulfide, methylmercaptan, fatty acids and in particular the trypsin-like protease, more recently referred to as gingivian (Shah et al., 1989). The gingivian has been shown to cause *in vitro* destruction of connective tissue components such as collagen and fibronectin (Smalley et al., 1988).

Perhaps the major virulence factor of *P. gingivalis* contributing to periodontal tissue destruction is the ability to induce bone resorption (Nair et al., 1983; Iino and Hopps, 1984; Hanazawa et al., 1985; Bom-Van Noorloos et al., 1986; Millar et al., 1986). However, it is still controversial whether or not this is

due to its LPS directly. Bom-Van Noorloos et al. (1990) investigated culture supernatants obtained from human PBMC in the presence of whole cell *P. gingivalis*. These supernatants were shown to contain a high concentration of bioactive and immunoreactive IL-1 which was responsible for strong osteoclast stimulating activity. Walsh et al.(1989) also reported *P. gingivalis* to be a potent inducer of the synthesis and secretion of IL-1 from monocytes when compared with the other plaque bacterium, *F. nucleatum*. Furthermore, Lindeman et al. (1988) demonstrated that *P. gingivalis* also induced another cytokine, tumor necrosis factor-α (TNF-α) which also has the ability to increase osteoclastic bone resorption. In contrast, resorbing bone cultures themselves did not react to the addition of bacteria (Bom-Van Noorloos et al., 1986;1989).

2.3. Immunological aspects of CIPD

2.3.1 Introduction

It has long been noticed that some individuals with large plaque accumulations and gingivitis for many years do not develop periodontal breakdown, whereas in others with little detectable plaque or gingivitis, progressive loss of periodontal tissues occurred (Van Palenstein Helderman, 1981). Furthermore, several investigators have reported that certain subjects with destructive periodontal diseases respond less well to conventional forms of periodontal therapy, including scaling and root planing, periodontal surgery and the administration of systemic antibiotics. They also noted that despite repeated seemingly appropriate therapy, multiple sites in certain subjects continue to show attachment loss over prolonged

period of time (Hirschfeld and Wasserman, 1978; McFall, 1982; Loesche et al., 1984; Lundstrom et al., 1984; Gordon et al., 1985). Such variation between individuals in terms of the severity of disease as well as in the response to periodontal treatment, supports the important role of host defence mechanisms in CIPD.

Early phenotypic and functional immunological studies have contributed a great deal towards an understanding of the immunological responses occuring in periodontal disease. A phenotypic characterization of immunocompetent cells in periodontal lesions, using enzymes and surface antigen markers, has been carried out. These studies have shown that the putative stable lesion is dominated by T-cells, while the progressive lesion is dominated by B-cells (Seymour and Greenspan, 1979a; Seymour et al., 1983).

Daly et al. (1983) illustrated that the predominant lymphocytes in chronically inflamed gingiva were T-cells with mean proportion of 53.9% while the minority (32.7%) was B-cells. In addition Arnitt (1986) showed that gingivitis (the putative stable lesion) in children is T-cell in nature. Further support for the predominantly B-cell character of the progressive lesion (Reinhardt et al., 1988) comes from the consistent finding of large number of plasma cells (Davenport et al., 1982) and immunoglobulin bearing lymphocytes (Mackler et al., 1977).

Hence, the conversion of a stable lesion to a progressive lesion may involve a change from a predominantly T-cell response to one involving large numbers of B-cells and plasma cells. This concept has been supported by a series of elegant studies by Taubman et al.(1981,1983) using rats sensitized to and then fed ovalbumin. They confirmed a shift from a T-cell to a B-cell dominated lesion as the lesion progressed to a more destructive stage. Yoshie et al.(1985) further showed that athymic nude rats manifested increased alveolar bone loss and the lesions contained predominantly B-cells while rats reconstituted with T-cells showed decreased bone loss. In these reconstituted animals T-cells predominated in the lesions, thus highlighting the role of T-cells in regulating the response.

2.3.2 Immunoregulation of CIPD by T-cells

Recently, most work on the immunoregulation of CIPD has been focusd on T-cells. As mentioned before, while immunohistology has shown a predominance of B-cells and plasma cells in advanced lesions, it is generally believed that regulation of this response is primarily mediated by T-cells (Seymour, 1987). Early phenotypic studies on cells extracted from progressive lesions in humans showed a depressed CD4:CD8 ratio (approximately 1:1) when compared to those from stable lesions or peripheral blood of healthy subjects (approximately 2:1) (Taubman et al., 1987). At the same time, functional studies have shown that these extracted cells from progressive lesions have a reduced ability to respond in an autologous mixed lymphocyte reaction (AMLR) (Cole et al., 1986).

Previously, CD4 and CD8 surface phenotypes were thought to relate to functional activity of T-cells. Helper functions were associated with the CD4 surface maker and cytotoxic/suppressor functions with the CD8 marker (Naor, 1992). It is now known that helper T-cells are found in both subsets and several studies have reported CD4+ cytotoxic and suppressor cells. Nevertheless, it has been established that both helper and cytotoxic/suppressor CD4+ T-cells recognize antigen in association with class II MHC molecules, while CD8+ T-cells also irrespective of their function recognize antigen in association with class I MHC determinants (Naor, 1992).

When the immune system encounters an antigen, it mounts a specific reaction that ultimately eradicates the infection. Antigen is first taken up by antigen-presenting cells (APC), processed, and expressed on the cell membrane in association with class I or class II MHC molecules depending on endogenous or exogenous antigens, which can then be recognized by CD8+ or CD4+ T-cells, respectively. The interaction leads to the production of growth and differentiation factors by both the APC and the T-cell, which in turn are responsible for the rapid expansion and differentiation of the initially small numbers of antigen-specific lymphocytes present in a previously unchallenged host (Swain, 1991). The antigen specificity and regulatory role of these cells in pathogenesis of CIPD is now a focus of attention. In human, limiting dilution analysis has demonstrated the presence of peripheral blood T-cells that are specific to *Porphyromonas gingivalis* and *Actinomyces viscosus* (Mahanonda et al., 1989). Initial treatment including scaling

and root planing, or removal of antigenic load, resulted in a substantial reduction in the proliferating T-lymphocyte precursor (PTL-P) frequencies of these cells (Mahanonda et al., 1991). In adult periodontitis subjects the median peak PTL-P frequency for *P. gingivalis* was 87.76×10^{-6} (approximately 1 in 12,000 T-cells) before treatment and this was reduced to 36.17×10^{-6} (approximately 1 in 28,000 T-cells) after treatment. In this group there was no change in the median peak PTL-P frequency for *A. viscosus* as a result of treatment. In contrast, in the gingivitis subjects, treatment led to a reduction in the PTL-P frequency for both *P. gingivalis* and *A. viscosus*. These data suggest *P. gingivalis* specific T-cells may be involved with antigen in the tissues of adults periodontitis subjects, while both *P. gingivalis* and *A.viscosus* specific T-cells may be involved in antigen in the tissues of gingivitis subjects. Direct evidence for a role of antigen specific T-cells in CIPD is however still lacking.

In order to explore the concept of antigen specificity in CIPD further, human peripheral blood T-cell lines responsive to antigens of *P. gingivalis* and *F. nucleatum* from periodontally healthy subjects have been developed (Ishii et.al., 1992). All the T-cells in these lines had the αβ T-cell receptor. The *P. gingivalis*-specific lines showed approximately equal proportions of CD4+ and CD8+ cells whereas the *F. nucleatum*-specific line was predominantly CD4+. The expression of CD25, HLA-DR, CD45RA, and CD29 varied throughout the 45 day culture period. A number of clones have been established from these cell lines. Characterization of these clones has shown that they are all CD4+ with a high level

of CD2, CD11a/CD18, CD29, CD45RO and HLA-DR expression. Conversely, they have low levels of CD45 RA, CD25 and CD54, which is suggestive of a memory phenotype. Upon antigen activation, while the level of CD45RO and CD29 expression remain stable, there is an upregulation of CD45RA which is consistent with the finding of a large number of CD45RA+, CD29+, CD4+ cells extracted from adult periodontitis lesions (Taubman et al., 1991).

The response of these T-cell clones is MHC class II restricted, they exhibit helper activity for B-cells as determined by their ability to induce high levels of IgG production in the presence of antigen and in general show a high level of IL-4 activity. Two of the clones also showed significant IFN-γ production in addition to the IL-4, while one showed both IL-4 and high levels of IL-2. The cytokine profile of these T-cell lines and clones revealed the mixture of Th1 and Th2 cytokines or on the other hand Th0 cytokine. These cell lines and clones were derived from the peripheral blood of periodontally healthy subjects. It is now necessary to establish cell lines and clones not only from peripheral blood of periodontally diseased subjects but also, if possible, with cell lines and clones derived from CIPD lesions.

2.3.3 Type 1 and type 2 T-cells

Recently, it has been demonstrated that functional subsets of T-cells which could not be distinguished by their surface phenotype as mentioned earlier, could be defined by their cytokine profiles. Mosmann and Coffman (1989) originally

described in the mouse, the type1 CD4+ T-cells were responsible for delayed type hypersensitivity (DTH) and secreted IL-2 and IFN-γ while the type 2 CD4+ T-cells provided B-cell help and produced IL-4, IL-5 and IL-6. These cells were refered to as Th1 and Th2 respectively. A third category of Th cell was also described. This cell can produce both Th1 and Th2 cytokines and have been designated Th0 cells (de Waal Malefyt et al., 1992). Recent studies by Bloom et al. (1992) have shown that similar cells exist in humans. The analysis has also been extended to CD8+ T-cells such that cytotoxic T-lymphocytes (CTLs) produce IFN-γ but not IL-4 while non- CTL CD8+ T-cell clones essentially produce a type 2 cytokine pattern i.e. IL-4 but not IFN-γ. Bloom et al. (1992), therefore suggest that functional T-cell subsets, irrespective of surface phenotype, be refered to as type1 or type2 on the basis of their cytokine profile (Seymour et al., 1983 and Bloom 1992) (Table 1)

In this model the primary function of type 1 CD4+ T-cell is DTH, while the secondary function is B-cell suppression. These cells primarily see antigen presented by macrophages and to a lesser extent by B-cells. Type1 CD8+ T-cells see antigen in a MHC classI restricted fashion on the surface of putative target cells, such as fibroblasts, and they function primarily as cytotoxic T-lymphocytes. Their secondary function is also B-cell suppression. In contrast the major antigen presenting cell for type2 CD4+ and CD8+ cells is a B-cell. The type2 CD4+ cells then provide B-cell help for specific antibody production with a secondary role of suppressing DTH. However, the primary role of the type2 CD8+ cell is DTH suppression and providing B-cell help is a secondary function.

Table 1 : Functional distinction of T-cells based on cytokine profile

(Modified by Seymour et al., 1983 and Bloom, 1992)

T-cell type	Phenotype	Antigen presenting cell	Cytokine profile	Major function	Secondary function
type 1	CD4	MØ (B-cell)	IL-2 IFN-γ	DTH	B-cell suppression
	CD8	Target cell (e.g. fibroblast)	IL-2 IFN-γ	CTL	B-cell suppression
type 2	CD4	B-cell MØ	IL-4 IL-5,IL-6	B-cell help	DTH suppression
	CD8	B-cell MØ	IL-4 IL-5,IL-6	DTH suppression	B-cell help

MØ = macrophage

DTH = delayed type hypersensitivity

CTL = cytotoxic T-lymphocyte

The immune response to infection would appear to be regulated by the balance between Type1 and Type2 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 naive T-cells are believed to progress into Th0 cells which secrete a combination of Type1 and Type2 cells and then differentiate further into Type1 or Type2 cells with repeated antigen stimulation (Modlin and Nutman, 1993). Differentiation of Th0 into Type1 cells is dependent on IFN-γ whereas differentiation into Type2 cell is dependent on

the presence of IL-4. These two cytokines reciprocally inhibit the induction of the other T-cell subset.

2.3.4 Cytokines

Cytokines are soluble low molecular weight proteins that produced by a wide range of cells and play important roles in many physiological responses. They are proteins involved in immune regulation. They are usually produced transiently. They are extremely potent, generally acting at picomolar concentrations and interact with high-affinity specific cell surface receptors, that are usually expressed in relatively low numbers. Individual cytokines have multiple overlapping cell regulatory actions. They interact in a network. The response of a cell to a given cytokine depends on the local concentration, the cell type, and other cell regulators to which it is constantly exposed.

Interleukin-2 was first discovered in 1976 (Morgan et al., 1976) and, since then, more than 20 cytokines have been characterized that are essential for many of the proliferative and differentiative functions of immune cells. Most have a variety of effects on many different cells by despite this overlap, cytokine functions are not identical.

As previously stated, Type1 T-cells produce IL-2 and IFN-γ, whereas Type2 T-cells secrete IL-4, IL-5 and IL-6. Following this concept, numerous attempts were made to define the patterns of cytokine production in T-cells stimulated in different

ways, and in cells isolated from experimental animals and from human during the course of normal and pathological immune responses. Detection of IFN-γ is associated with the Type1 T-cells, whereas detection of IL-4 is associated with Type2 T-cells.

2.3.4.1 The interferons

Interferons are inducible proteins which are important not only in defence against a wide range of viruses but also in the regulation of immune responses in haematopoietic cell development (Langer,1985). IFN-α and IFN-β are produced by most cells in response to viral infection or stimulation with natural or synthetic double-stranded RNA. They can enhance the expression of class I MHC gene products and enhance natural killer (NK) cell activity and thus play a role in immunoregulation. IFN-γ has potent immunoregulatory effects on a variety of cells including activation of macrophages, enhanced production of IgG2a by B-lymphocytes, and enhanced expression of Class I and Class II MHC gene products. IFN-γ counteracts the effects of IL-4 on B cells and thus inhibits the activity of Th2 cells. It is produced by T lymphocytes from blood or lymphoid tissues upon stimulation with specific antigens, mitogens, or allo-antigens. Both CD4+ and CD8+ T lymphocytes and NK cells can produce IFN-γ , although the Th1 CD4+ cells are considered the major producers in response to antigens (Mosmann,1989).

2.3.4.2 Interleukin-4 (IL-4)

IL-4 is produced by T-cells, mast cells, and basophils (Sher et al., 1992) and is an important factor in the clonal expansion of antigen-specific B-cells. Therefore it would appear that IL-4 modulates humoral responses to different antigenic stimuli. IL-4 contributes to negative immune regulation by its ability to reduce IL-2 receptors and thus inhibiting some IL-2 induced activities (Gemmell, 1994a).

2.3.5 Cytokine profiles of gingival cells

A number of studies have reported on the presence of cytokines in periodontal disease. Pilon et al. (1991) demonstrated lower levels of IL-2 in gingival crevicular fluid of periodontal disease sites compared with that from healthy sites while Fujihashi et al. (1991) have shown that gingival mononuclear cells from adult periodontitis patients produce IL-4, IL-5, but not IL-2. Gemmell and Seymour (1994a) have shown a significantly less IL-2 activity in peripheral blood mononuclear cell cultures stimulated by both *Porphyromonas gingivalis* and *Fusobacterium nucleatum* than in unstimulated cultures. In this study IFN-γ as measured by an immunoassay could not be detected in cultures containing both bacteria and furthermore, IFN-γ was demonstrated in only 10 out of 27 gingival mononuclear cell culture supernatants. Only negligible amounts were found in 50% of these positive cultures. Taken together, these results indicate possible lower levels of both IL-2 and IFN-γ in progressive periodontitis lesions, suggesting decreased Th1 responses and a possibility of increased Th2 responses.

2.3.6 Immunopathogenesis of CIPD: hypothesis

As mentioned, previous studies have established the B-cell nature of the progressive lesion. Non-specific polyclonal B-cell activation (PBA) has been cited as one possible mechanism and a number of oral bacteria including *F. nucleatum* have been shown to be polyclonal B-cell activators. However, in human PBA is regulated by T-cells. Indeed, there is a requirement for IL-4 for B-cell proliferation and differentiation and as previously described there is strong evidence, albeit circumstantial, that T-cell activation in CIPD is antigen specific. Hence, it is possible that in susceptible subjects the "gingiva-homing" T-cells may be predominantly IL-4 producing type 2 cells, the CD4 subset of which would provide B-cell help. In the presence of PBA (possibly due to a second organism such as *F. nucleatum*) there would be an expansion of the B-cell population with the production of large amounts of B-cell IL-1 which would then, mediate tissue destruction via a number of pathways. As this B-cell population expanded, there would be an increase in the number of antigen-specific B-cells which in turn would be capable of presenting antigen, leading to specific antibody production. If this was protective antibody it could result in elimination of the organism and disease progression would stop. Anti-idiotypic networks could then turn off the B-cell response. If the antibody was not protective, continuous connective tissue breakdown could ensue. The type2 CD8+ cells would have as their primary function, suppression of DTH which in this context is suppression of the putative T-cell stable lesion. In this model therefore the period of destruction would proceed the period of stability and the disease may have a cyclical pattern with not all

B-cell lesions being destructive. The type 2 T-cell therefore could be seen to have both a protective (via specific antibody) and destructive (via PBA and IL-1 production) elements.

In non-susceptible subjects type1 IL-2 and IFN-γ producing T-cells may home preferentially to the gingiva. The type1 CD4+ T-cell subset would then induce a DTH type response which may be synonymous with the putative T-cell stable lesion. The CD8 population may represent a CTL population which could lead to fibroblast cytotoxicity and the subsequent imbalance in tissue remodelling resulting in a net loss of collagen and hence a relatively slow progression of the disease. The secondary function of both the CD4+ and CD8+ type1 T-cells is suppression of the B-cell lesion.

Chronic inflammatory periodontal disease however, must be seen to be multifactorial in etiology and pathogenesis. While this model may explain in part, differences in immune responses, the disease process would also be influenced by bacterial virulence factors and by environmental factors such as stress.

This model is still speculative. It could be that the type 1 T-cell is associated with the non-susceptible subject and the type 2 T-cell is indicative of susceptibility. Either way, by testing this model experimentally process towards an understanding of why some people lose their teeth while others do not will be

inevitable. In order to test the model, studies would need to utilise antigen specific T-cells such as *P. gingivalis* specific T-cells. This could be done by establishment of T-cell lines reactive to periodontopathic bacteria from peripheral blood of periodontally diseased subjects and followed by analysis of cytokine profiles of these cells so as to determine their role in the pathogenesis of CIPD.