

# The immunopathogenesis of periodontal disease

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## ABSTRACT

Treatment planning in periodontics, as with any disease, must be based on an understanding of the aetiology and pathogenesis of the disease. In this context, it has slowly become recognized over the past three decades that while plaque is the cause of the disease, it is the innate susceptibility of the host that determines the ultimate outcome of the disease process. Innate susceptibility, in turn, is determined by the nature of the immune response to the specific periodontopathic complexes comprising the plaque biofilm. The aim of this review was to examine current understanding of the immunopathogenesis of chronic periodontitis with respect to its possible clinical implications in terms of treatment planning and risk assessment. Numerous studies have demonstrated that the periodontitis lesion itself involves predominantly B cells and plasma cells, while the gingivitis lesion is primarily a T cell mediated response. This led to the concept over 30 years ago that the development of periodontitis involves a switch from a T cell lesion to one involving large numbers of B cells and plasma cells. It is also well recognized that control of this shift is mediated by a balance between the so-called Th1 and Th2 subsets of T cells, with chronic periodontitis being mediated by Th2 cells. More recently, T regulatory (Treg) and Th17 cells have been demonstrated in periodontal tissues, raising the possibility that these cells are also important in the immunoregulation of periodontal disease. The clinical implications of these observations can be seen in the fact that identification of Th1/Th2 and Treg/Th17 cytokine gene expression in the peripheral blood and salivary transcriptomes is now being trialled as a possible marker of disease susceptibility. If this proves to be the case, a chairside salivary diagnostic could be developed within the next five to 10 years.

**Keywords:** Aetiology, chronic periodontitis, immunopathogenesis, pathogenesis, susceptibility.

**Abbreviations and acronyms:** DTH = delayed type hypersensitivity; GCF = gingival crevicular fluid; MMP = matrix metalloproteinases; PMN = polymorphonuclear leukocyte; TLR = toll-like receptor; TNF- $\alpha$  = tumour necrosis factor- $\alpha$ .

## INTRODUCTION

Treatment planning in periodontics has traditionally been based on a detailed assessment of probing depths, attachment levels, furcation involvement, mobility, occlusal abnormalities, habit patterns, and mucogingival defects among others. Individual teeth and the dentition as a whole are then typically given a prognosis, which is the clinician's best guess of their fate. However, a landmark study by Hirschfeld and Wasserman<sup>1</sup> clearly demonstrated just how inaccurate this approach is. In this study, the outcomes after 20 years of periodontal treatment were analysed. Despite similar treatment approaches being applied to all patients based on prognosis and risk factors, distinct groups of patients were identified as displaying remarkably different disease courses. The so-called "well-maintained" group lost only 17.1 per cent of teeth that were originally classified as "questionable", while the so-called "extreme downhill" group lost

88.4 per cent of teeth that were originally classified as questionable. Across all groups however, only 31 per cent of teeth that were given a poor prognosis 20 years earlier were actually lost, while a significant number of teeth that were initially given a favourable prognosis were ultimately lost. In fact, in the "extreme downhill" group, over half the teeth lost were initially given a favourable prognosis. This "extreme downhill" group can therefore be said to have been highly susceptible. Importantly, these workers clearly showed that it was patient susceptibility that determines the ultimate outcome, rather than a history of previous attachment loss, probing depths etc. The study highlights the importance of innate susceptibility in the pathogenesis of periodontal disease.

Experimental gingivitis studies in the 1960s<sup>2</sup> elegantly demonstrated that gingivitis is the response of the body to the build-up of dental plaque. These studies also showed that there is individual variation in this

response with some individuals taking longer to manifest disease compared with others. So, while it has been known for many years that plaque is the aetiological agent, the factors contributing to patient susceptibility are still not fully understood. Not all individuals with gingivitis will progress to periodontitis, and not all individuals with periodontitis will progress to tooth loss. The difficulty arises in identifying those with disease expression who will go on to experience disease progression.

Throughout the 1980s, much emphasis was placed on the identification of specific periodontal pathogens based on the concept that the presence of these pathogenic organisms predicts disease outcomes. However, in a landmark five-year longitudinal study in the 1990s, Cullinan *et al.*<sup>3</sup> showed that there is great volatility in the acquisition and loss of these organisms, as many people who carry the putative pathogens do not manifest disease. In other words, patient susceptibility together with the presence of specific periodontal pathogens, will determine the ultimate disease outcome. Superimposed on this are environmental factors such as smoking and stress which impact on disease expression and progression via their effect on the way in which the host responds to the periodontopathic bacterial complexes.

### The development of gingival inflammation

Accumulation of plaque at the gingival margin results in the development of gingivitis<sup>2</sup> and in susceptible individuals, this will progress to periodontitis.<sup>4</sup> Figures 1, 2 and 3 illustrate this progression. The development of gingivitis and periodontitis can be loosely divided into a series of stages as described by Page and Schroeder.<sup>5</sup> These authors classified the development of the disease into the initial, early, established and advanced lesions.



Fig 1. Healthy gingiva.



Fig 2. Severe gingivitis (stable lesion).

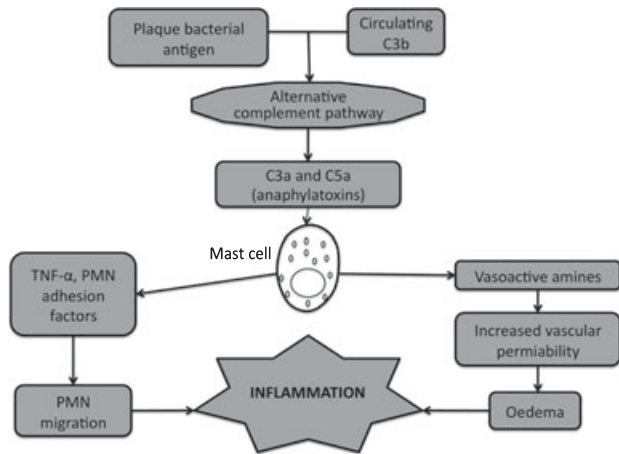


Fig 3. Chronic periodontitis (progressive lesion).

### The initial lesion

The initial lesion occurs within the first four days following the beginning of plaque accumulation. It is a subclinical lesion that can only be observed histologically but is characterized by the formation of oedema, an increase in gingival fluid flow, an accumulation of polymorphonuclear leukocytes (PMNs) and loss of connective tissue. As plaque accumulates, bacterial enzymes and metabolic end products increase the permeability of the junctional epithelium, allowing both the ingress of further bacterial products and at the same time the outflow of gingival fluid. This gingival fluid is essentially a serum product, which contains all the components of complement.

Activation of complement via the so-called “alternative pathway” in the gingival sulcus results in production of the anaphylatoxins C3a and C5a, which in turn lead to the release of vasoactive amines from mast cells. These vasoactive substances lead to an increase in vascular permeability and the formation of oedema, one of the hallmarks of inflammation (Fig 4 illustrates



**Fig 4.** A flow chart representing the interaction of plaque bacterial antigen, the complement system and mast cells that leads to inflammation. Circulating C3b interacts with plaque derived bacterial antigen. Through the alternative complement pathway, an amplification loop is established that results in formation of large quantities of the anaphylatoxins C3a and C5a. Mast cells are stimulated by C3a and C5a, leading to release of TNF- $\alpha$ , PMN adhesion factors and vasoactive amines. This results in PMN chemotaxis and the development of oedema which together define inflammation.

this process). Also at this initial stage, the mast cells release preformed tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), which is largely responsible for the expression of adhesion molecules by endothelial cells and the subsequent sticking and migration of PMNs into the gingival tissues and out into the gingival sulcus. While activation of the alternative complement pathway is essential for the vascular responses, bacterially derived chemotactic substances together with C5a are responsible for the initial migration of PMNs. Once in the gingival sulcus however, the PMNs are unable to phagocytose the bacteria, which are now forming a biofilm and as such are firmly adherent to the tooth surface. In this situation, the PMNs discharge their lysosomal contents into the gingival sulcus in what has been termed "abortive phagocytosis". These lysosomal enzymes can then get back into the tissues and contribute to the local destruction of connective tissues. At this initial stage, the lesion occupies no more than 5–10 per cent of the connective tissues,<sup>5</sup> and is still not clinically evident.

### **The early lesion or stable lesion**

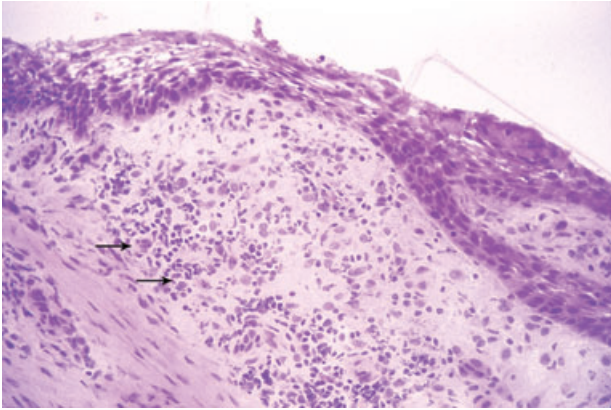
At approximately 4–7 days of plaque accumulation, the nature of the developing lesion changes from one consisting primarily of PMNs to one with increased numbers of lymphocytes and macrophages. This is called the early lesion in which vascular changes become more pronounced as illustrated by the activation of previously dormant capillary beds, and the development of perivascular inflammatory infiltrates. As a result, there is a net increase in the flow of fluid

into the affected gingival tissues, and a subsequent increase in the flow of gingival crevicular fluid. Further concurrent widening of intercellular spaces between the epithelial cells of the junctional epithelium allows increased diffusion of bacterial products into the gingival tissues and escalation of the inflammatory response.

The lesion begins as small perivascular infiltrates which progressively increase in size and coalesce until they become clinically evident at around day 12 to 21. By day 21, lymphocytes make up 70 per cent of the infiltrate with PMNs and plasma cells making up less than 10 per cent of the total infiltrate within the tissues.<sup>6</sup> However, PMN numbers increase four-fold within the junctional epithelium.<sup>7</sup> Increases in cell adhesion molecules such as endothelial cell leukocyte adhesion molecule-1 (ELAM-1) and intercellular adhesion molecule-1 (ICAM-1), together with an increase in Interleukin-8 (IL-8) production by the epithelial cells, help to establish a fast flow of PMNs through the junctional epithelium and into the gingival sulcus,<sup>8</sup> where they form a barrier against plaque microorganisms.<sup>9</sup> Although the infiltrated area remains fairly localized at this stage, up to 60–70 per cent of collagen within the infiltrated zone is degraded.<sup>5</sup>

The immunological events occurring during the development of gingivitis have been described by Seymour *et al.*<sup>10</sup> As noted above, gingivitis develops as perivascular lymphocyte/macrophage lesions. As these increase in size, they coalesce and merge together, eventually becoming clinically evident. The lymphocytes are predominantly T cells with a CD4:CD8 ratio of around 2:1. The cells are activated, and along with sulcular epithelial cells, express high levels of MHC class II antigens (HLA-DR and HLA-DQ). Increased numbers of Langerhans cells are seen in the oral as well as oral sulcular epithelium. Throughout the development of gingivitis, less than 5 per cent of the T cells express the IL-2 receptor CD25, suggesting that these cells are not proliferating locally. While interdigitating dendritic cells can be found in the perivascular spaces, the majority of macrophages in the developing lesion are acid phosphatase positive phagocytic cells. This sequence of events is identical to that seen in the development of delayed type hypersensitivity (DTH)<sup>11</sup> (Fig 5).

As soluble antigen enters the tissues, it is taken up by the resident Langerhans cells and carried to the regional lymph nodes where antigen specific T cells are sensitized. These sensitized cells then travel back to the site of original antigen challenge (i.e., the gingival tissues). Once there, following further antigen presentation by dendritic cells, they become activated and together with the infiltrating phagocytic macrophages they control the ingress of antigen and achieve a balance with the plaque biofilm.

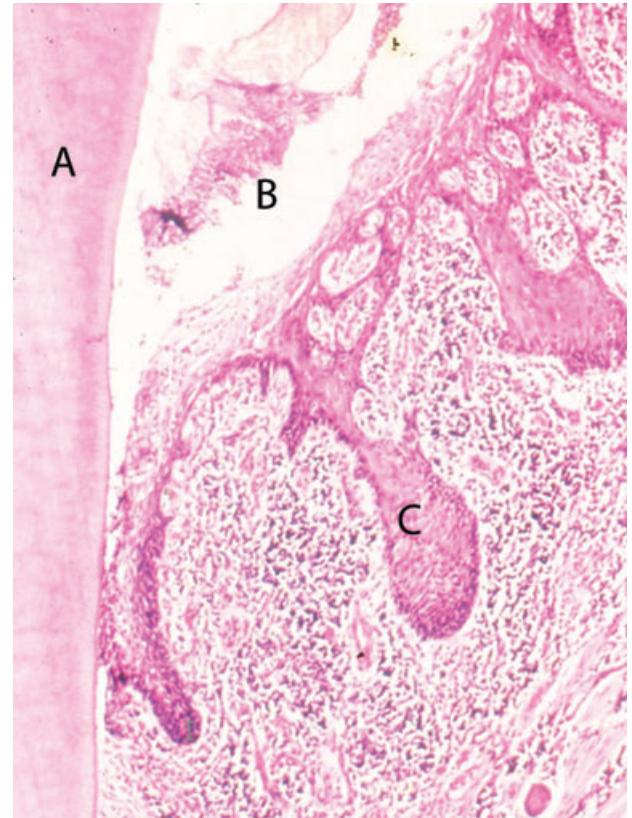


**Fig 5.** Delayed type hypersensitivity (DTH). A perivascular lymphocyte/macrophage infiltrate is seen within the connective tissue, which is characteristic of DTH. Arrows point to capillaries in cross section with surrounding infiltrate.

The development of DTH is a well-controlled immunological response which develops in 12–24 hours, peaks within 48 hours and is gone after a week. In this context, gingivitis can also be considered to be a well-controlled immunological response but because of the persistence of the plaque biofilm, the immunological response persists rather than resolving. Because plaque bacteria only rarely invade the host tissues, the various phagocytes (PMNs in the gingival sulcus and macrophages in the tissues) are unable to eradicate the microbial challenge. The subsequent, prolonged nature of the inflammatory response results in gingivitis becoming chronic in nature. While in most people the immune response is able to contain the microbial challenge, it is only with mechanical cleaning that the microbial challenge can be eradicated. Collagen is degraded in the stable lesion but does not result in any loss of attachment. When the plaque is removed, gingival tissues repair and remodel, and there is no permanent damage or alteration of tissue architecture.

### **The established or progressive lesion**

In some people, either due to environmental factors, their own innate susceptibility, or both, the stable lesion changes to a B cell/plasma cell response with the production of high levels of Interleukin-1 (IL-1) and Interleukin-6 (IL-6) and subsequent connective tissue breakdown and loss of bone. As the connective tissue attachment to the tooth breaks down, the junctional epithelium migrates in an apical direction and a periodontal pocket forms, which becomes lined by pocket epithelium with in-growth of rete pegs into the surrounding connective tissue (Fig 6). Increased permeability of this pocket epithelium allows continued ingress of microbial products, the continued production of inflammatory cytokines such as IL-1, TNF- $\alpha$ , and Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>),<sup>12</sup> and perpetuation of the



**Fig 6.** A histological section of a gingival sulcus exhibiting signs of inflammation. (A) is the root surface; (B) is the gingival sulcus and; (C) is an elongated rete peg.

inflammatory process leading to continued tissue destruction.<sup>13</sup> The main identifying feature of the progressing, established lesion is the predominance of plasma cells within the periodontal connective tissues<sup>14–16</sup> indicative of a B cell adaptive immune response.<sup>17</sup>

### **The advanced lesion**

The advanced lesion has essentially the same cellular make-up as the established lesion. The main difference lies in the overt loss of attachment that is evident clinically and histologically. It is now generally accepted that the mechanism of tissue destruction is via the effects of the immune response.<sup>18</sup> Fibroblasts and macrophages are stimulated by the inflammatory cytokines IL-1, TNF- $\alpha$  and PGE<sub>2</sub> to produce matrix metalloproteinases (MMP),<sup>19,20</sup> which are a family of proteinases whose primary purpose is the degradation of the extracellular matrix.<sup>21</sup> Collagen molecules are cleaved into smaller fragments, which then become denatured in the extra-cellular environment or are phagocytosed by surrounding fibroblasts. As the lesion advances, alveolar bone loss becomes apparent. However, a non-infiltrated fibrous band remains adjacent to the crestal bone, which effectively encapsulates the progressing lesion.

## Immunoregulation

As stated above, in some people, either due to environmental factors, their own innate susceptibility, or both, the stable T cell lesion changes to a B cell/plasma cell response with the production of high levels of IL-1, IL-6 and PGE<sub>2</sub> and subsequent connective tissue breakdown and bone loss. Therefore, in this context, understanding the regulatory mechanisms involved is fundamental to understanding susceptibility to periodontitis.

The nature of the adaptive immune response is dependent on a complex interplay between various immunological networks. T cells are central in the control of immune mediated mechanisms and in this context, the balance between the so-called Th1 and Th2 cells is crucial. T helper 1 (Th1) and T helper 2 (Th2) cells were first described by Mosmann in 1986.<sup>22</sup> Th1 cells mediate predominantly cell mediated immune responses, as demonstrated by DTH, by secreting Interleukin 2 (IL-2) and Interferon gamma (INF- $\gamma$ ). The secondary function of Th1 cells is the suppression of B cells and plasma cells. In contrast, Th2 cells induce predominantly B cell humoral immune responses by secreting Interleukin 4 (IL-4), Interleukin 5 (IL-5) and Interleukin 10 (IL-10) while their secondary function is the suppression of T cell mediated responses.<sup>23</sup> Therefore, immunoregulatory control depends upon the balance between these two T cell subsets.

The fact that the development of gingivitis is identical to the development of DTH and that progressive chronic periodontitis is fundamentally a B cell lesion, led to the concept that gingivitis, and hence the stable periodontal lesion, is mediated by Th1 cells, while on the other hand chronic periodontitis is mediated by Th2 cells.<sup>24</sup> In this concept, it is proposed that a strong innate immune response leads to the production of high levels of IL-12 by both PMNs and macrophages which in turn leads to a Th1 response, cell mediated immunity, protective antibody and a stable periodontal lesion. In contrast, a poor innate immune response with polyclonal B cell activation leads to a Th2 response, non-protective antibody and a progressive periodontal lesion.

Since being put forward almost 15 years ago, this hypothesis has attracted a lot of attention with a number of studies supporting the hypothesis showing either depressed Th1 responses or increased Th2 responses in chronic periodontitis. In contrast, other studies (primarily in animal models) have implicated increased Th1 responses in chronic periodontitis, while others have highlighted a role for Th0 cells. Nevertheless, it is now generally agreed that chronic periodontitis in humans is mediated by Th2 cells.<sup>25,26</sup>

## What determines the nature of the immune response?

While the Th1/Th2 paradigm provides a possible mechanism by which periodontal lesions become progressive or remain stable, an important question that remains is, what causes some lesions to show Th1 characteristics while others show Th2 characteristics? The answers may lie in the nature of the microbial challenge as well as particular genetic and environmental susceptibility factors. Importantly, some of these factors may be clinically identifiable and modifiable.

### Genetics

Innate individual susceptibility to chronic periodontitis may involve both genetic and environmental factors. Twin studies have indicated a substantial genetic basis to chronic periodontitis.<sup>27,28</sup> In a series of experiments, using different strains of mice, Gemmell *et al.*<sup>29-33</sup> showed that certain strains of mice (Balb/c and DBA/2) are susceptible to *P. gingivalis* infection whereas others (CBA and C57/bl) are resistant. In these experiments, the susceptible strains also showed low Th1 responses while the resistant strains showed moderate to high Th1 responses to *P. gingivalis*. As well, mice with low susceptibility (i.e., resistance to disease) have high levels of IgG2a (Th1) and low levels of IgG1 (Th2). These results suggest that genetics (H-2 in mice or HLA in humans) may in part determine the cytokine and antibody profile and hence susceptibility to disease.

Over the last decade, a large number of gene polymorphisms have been identified as being associated with increased periodontal disease susceptibility. At this stage, investigations into the significance of these have yielded mixed results.<sup>34</sup> However, longitudinal studies<sup>35,36</sup> over five years showed that there is a direct interactive effect between smoking and disease, between age and disease, and between *P. gingivalis* and disease but there is no direct interactive effect between IL-1 genotype and disease. However, in this study, IL-1 genotype positive subjects with *P. gingivalis* had 80 per cent more disease than IL-1 genotype negative subjects with *P. gingivalis*, and IL-1 genotype positive smokers had 70 per cent more disease than IL-1 genotype negative smokers. Significant interactive effects were also found between smoking and IL-10 genotype. In this context, smoking, age and the presence of *P. gingivalis* can be seen as primary risk factors while IL-1 and IL-10 gene polymorphisms can be viewed as secondary risk factors, having a significant effect only in the presence of a primary risk factor.

### Nature of the microbial challenge

There is no doubt that plaque is the sole aetiological agent for gingivitis and periodontitis. Over the past

decade however, biofilms containing complexes including *Porphyromonas gingivalis*, *Tannerella forsythia*, *Aggregatibacter actinomycetemcomitans* and *Treponema denticola* have been related to chronic periodontitis,<sup>37</sup> such that it is unlikely that a single antigen or a single organism is responsible for the disease. Indeed, little is actually known of the biofilm specific antigens involved in periodontal disease and of the immune response to them. In 2000, Choi *et al.*<sup>38</sup> showed that T cell clones derived from mice immunized with *P. gingivalis* alone had a Th1 profile, whereas T cell clones derived from mice immunized with *Fusobacterium nucleatum* followed by *P. gingivalis* demonstrated a Th2 profile. This may be due to the fact that *F. nucleatum* is a polyclonal B cell activator such that B cells subsequently present the *P. gingivalis* antigen. Further, Gemmell *et al.*<sup>30,31</sup> showed that if mice were immunized with *F. nucleatum*, they were subsequently unable to make antibody to *P. gingivalis*. This was not the case if bacteria were injected in the reverse order. These results, albeit preliminary, nevertheless show that it is possible for co-infection to modulate the immune response. The level of this modulation remains to be demonstrated but it is likely to involve the Th1/Th2 balance.

In their five-year longitudinal study, Cullinan *et al.*<sup>3</sup> showed a direct effect between plaque complexes containing *P. gingivalis* and disease progression. No such effect was seen with complexes containing *A. actinomycetemcomitans*, nor *Prevotella intermedia*, such that these organisms were considered to be of only minor importance in periodontal disease progression. Nevertheless, it is possible that *P. gingivalis*, and hence complexes containing *P. gingivalis*, have the potential to modify the host response. In a recent study, albeit in mice, Gemmell *et al.*<sup>39</sup> showed, using microarray analysis, that *P. gingivalis* up-regulates only five genes compared with 1141 genes that were down-regulated in CD4 cells. Sixty of these genes are involved in the immune response. Similarly, CD8 T cells showed up-regulation of only 28 genes and down-regulation of 1175 genes, with 65 of these genes being involved in the immune response. This study highlights a powerful down-regulatory effect of *P. gingivalis* on the host immune response. Although the effects of these genes on the Th1/Th2 response is mixed, it may indicate a shift away from the Th1 response.<sup>39</sup> Therefore, it would appear that the nature of the microbial challenge may, at least in part, determine the nature of the immune response and hence progression of disease.

### Innate immunity

Innate immunity is a consistent feature of both gingivitis and periodontitis. A strong innate immune response, with high levels of IL-12, has been associated

with a Th1 response while a poor innate immune response has been suggested to favour a Th2 response. Recently, it has been shown that the levels of the active IL-12p70 are significantly higher in the gingival crevicular fluid (GCF) from gingivitis sites in both gingivitis and periodontitis patients compared with periodontitis sites from the same patients.<sup>40</sup> Although not significant, slightly lower levels of IL-12p40 were found in the GCF from periodontitis sites. IL-12p40 is produced primarily by activated PMNs, macrophages and dendritic cells. It can also be produced by keratinocytes and although it is a component of IL-12p70, it is generally thought to inhibit its activity by binding competitively to the IL-12 receptor IL-12Rβ1. However, recently it is increasingly being recognized as an independent cytokine, which not only acts as a chemoattractant for macrophages and promotes the migration of bacterially stimulated dendritic cells, but also is protective in a mycobacterial model.<sup>41</sup> In this context, the slightly higher levels of IL-12p40 seen in gingivitis might in fact support the protective Th1 response.

### Toll-like receptors

The discovery of toll-like receptors (TLRs) has led to a far greater understanding of innate immunity and the induction of adaptive immunity. TLRs are found on dendritic cells, neutrophils and macrophages among others and have the ability to recognize structures that are highly conserved across a wide variety of pathogens. Such structures include LPS, peptidoglycan, bacterial DNA, double stranded RNA and lipoprotein.<sup>42</sup>

Given their role in innate immunity, it is likely that TLRs are important in determining the nature of the host response to plaque. TLR-2 and TLR-4, upon stimulation, may induce markedly different immune responses as determined by the resulting cytokine profiles. When stimulated, TLR-4 has been shown to promote expression of IL-12p70 and INF-γ inducible protein-10 (IP-10), which is indicative of a Th1 response. Conversely, TLR-2 promotes the inhibitory IL-12p40, which is characteristic of a Th2 response.<sup>43</sup> These differences are reflected in differential cytokine expression by *E. coli* derived LPS and *P. gingivalis* derived LPS. *E. coli* derived LPS, which activates TLR-4 induces a strong Th1 response, while *P. gingivalis* derived LPS, which activates TLR-2,<sup>44</sup> induces a strong Th2 response.<sup>45</sup> These findings indicate a further mechanism of susceptibility to periodontitis.

### Autoimmunity in periodontal disease: the Treg/Th17 axis

While over the past two decades most attention has focused on Th1 and Th2 cells, in recent years a third

lineage of T cell has been described. These are the so-called Th17 cells, which selectively produce Interleukin-17 (IL-17). IL-17 induces the secretion of IL-6, IL-8 and PGE<sub>2</sub> hence these cells are thought to play a crucial role in regulating inflammation. IL-17 is also thought to affect osteoclast activity and thereby mediate bone resorption.

In the mouse, naïve T cells when incubated with transforming growth factor- $\beta$  (TGF- $\beta$ ) and IL-2 up-regulate the forkhead/winged helix transcription factor Foxp3 and develop into the so-called Treg cells, which have an important function in suppressing autoimmune responses. In contrast, when incubated in the presence of TGF- $\beta$  and IL-6, CD4<sup>+</sup> T cells express the transcription factor ROR $\gamma$ t and become Th17 cells. While these cells are thought to have a protective role against bacterial infections, they may on the other hand contribute to autoimmune disease. Activation of monocytes via TLR-2 is an effective stimulus for Th17 differentiation and while IL-2 initially inhibits Th17 differentiation, ultimately it leads to Th17 expansion.<sup>46</sup>

In addition to Th17 cells, CD4<sup>+</sup>CD25<sup>+</sup> regulatory T-cells (Tregs), infiltrate and may play a role in periodontal disease.<sup>47</sup> An immunohistological and gene expression study<sup>47</sup> has shown increased Tregs in periodontitis with increased proportions of B cells. Foxp3, a characteristic marker of Tregs, was also shown to be more highly expressed in periodontitis compared with gingivitis.

In the mouse gene array study, *P. gingivalis* led to the down-regulation of the IL-17 receptor (IL-17r) gene.<sup>39</sup> IL-17r deficient mice have a defect or display a significant delay in neutrophil recruitment into infected sites resulting in susceptibility to infection,<sup>48</sup> which may account partly for the reported inhibition of PMNs into the *P. gingivalis*-induced lesion in mice.<sup>29</sup> In contrast to the mouse study, IL-17 expression has been shown to be up-regulated in human periodontitis tissue.<sup>49</sup> This finding was supported by the gene expression profile of T-cell clones established from periodontitis patients where 51 per cent of gingival T-cell clones expressed IL-17 compared with only 11 per cent of peripheral blood T-cell clones.<sup>50</sup> As well, stimulation of peripheral blood mononuclear cells by *P. gingivalis* antigen enhanced not only transcription but also translation of the IL-17 gene.<sup>49</sup> As IL-17 is capable not only of inducing IL-6 in gingival fibroblasts, but also of enhancing the humoral immune response as well as the inflammatory response, the balance between the production of IL-17 and expression of its receptor further reflects the fact that cytokines cannot be studied in isolation and that it is the balance of cytokines that is fundamental in disease expression.

The role of autoimmunity in chronic inflammation is still not clear. It is possible that autoimmunity is a feature of all chronic inflammatory processes. In this

context it has been known for many years that gingival fibroblasts are able to phagocytose collagen such that anti-collagen antibodies may facilitate this phagocytosis and hence the removal of broken-down collagen. At the same time an anti-HSP response may enhance the removal of dead and dying cells such that these autoimmune responses may be a natural part of chronic inflammation. Control of these responses would therefore be essential. This concept illustrates that the role of T cells in periodontal disease may be one of immune homeostasis. Further studies are clearly needed to test this hypothesis and to determine the role of regulatory T-cells in periodontal inflammation.

### The role of the immune response in defining risk

While there is no doubt that patient susceptibility determines periodontal disease expression and that this in turn involves the interaction of aetiological, host and environmental factors, determination of risk in periodontics has proved elusive. Recently in a very preliminary study, Seymour *et al.* (unpublished data) asked the questions: can susceptible patients be identified on the basis of differential immune response gene expression; and can the salivary or peripheral blood transcriptome be used to identify susceptible patients?

These workers extracted total RNA from leukocytes isolated from the peripheral blood (i.e., the peripheral blood transcriptome) of a subject with gingivitis, and from a subject with periodontitis both before and after treatment. The pattern of gene expression was determined using Affymetrix GeneChip® U133 plus 2.0 Human Genome Array. Only genes involved in the immune response (as annotated by affymetrix) and where there was a minimum two-fold change (increase or decrease) in expression were considered.

In periodontitis, compared with gingivitis, 181 immune response genes were differentially expressed. Of these, 126 genes were up-regulated in periodontitis compared with gingivitis and 55 genes were down-regulated in periodontitis compared with gingivitis. Following non-surgical periodontal treatment, 53 immune response genes were differentially expressed, with 52 genes being down-regulated and only 1 gene, the IL-8 gene, being up-regulated after periodontal treatment. It must be emphasized however, that these are very preliminary results and at this stage it still remains to be determined if susceptible patients can be identified on the basis of differential gene expression but these preliminary results do offer some interesting prospects.

### CONCLUSIONS

Treatment planning in periodontics is no longer based on probing depths, mobility, occlusal abnormalities,

mucogingival defects etc., but rather is based on an understanding of the aetiology (plaque) and pathogenesis (patient susceptibility) of the disease. Susceptibility involves the interaction between host, bacterial and environmental factors and differential gene expression offers exciting prospects for identifying patients potentially at risk.

## ACKNOWLEDGEMENTS

Studies carried out by the authors and reported in this paper were supported in part, by the National Health and Medical Research Council of Australia, the Australian Dental Research Foundation, Colgate Oral Care Australia and Colgate Palmolive Ltd USA. Clinical studies were carried out in association with Bill Westerman, Margaret Roberts, Jan Palmer and Malcolm Faddy. Immunological and microbiological studies were in association with Erica Gemmell, Phil Bird and Steve Hamlet, while gene arrays were carried out together with Agnes Lichanska, Steve Hamlet, Andres Orozco and Rod Marshall.

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