Cytokines in periodontal disease: where to from here?

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Numerous studies have attempted to elucidate the cytokine networks involved in chronic periodontitis, often with conflicting results. A variety of techniques were used to study cells in situ, cells extracted from gingival tissues, peripheral blood mononuclear cells, purified cell populations, and T cell lines and clones. Bacterial components, including sonicates, killed cells, outer membrane components, and purified antigens, have all been used to stimulate cells in vitro, making comparisons of cytokine profiles difficult. As it is likely that different cells are present at different disease stages, the inability to determine disease activity clinically is a major limitation of all these studies. In the context of tissue destruction, cytokines such as IL-1, IL-6 and IL-18 are likely to be important, as are their regulating cytokines IL-10 and IL-11. In terms of the nature of the inflammatory infiltrate, two apparently conflicting hypotheses have emerged: one based on direct observations of human lesions, the other based on animal experimentation and the inability to demonstrate IL-4 mRNA in gingival extracts. In the first of these, Th1 responses are responsible for the stable lesion, while in the second Th2 responses are considered protective. Using *Porphyromonas gingivalis*-specific T cell lines we have shown a tendency for IFN-γ production rather than IL-4 or IL-10 when antigen is presented with peripheral blood mononuclear cells which may contain dendritic cells. It is likely that the nature of the antigen-presenting cell is fundamental in determining the nature of the cytokine profile, which may in turn open up possibilities for new therapeutic modalities. □ *Antigen presentation; cytokines; periodontal disease; Th1/Th2*

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Chronic inflammatory periodontal disease manifests clinically as at least two distinct entities. Evidence based on microbiological, immunological, and clinical studies has shown that some forms of periodontal disease in adults can remain stable over many years and not endanger the life of the dentition, whereas other forms, despite extensive treatment, continue to break down, leading ultimately to tooth loss (1). Periodontal disease is caused by bacteria in dental plaque, with evidence that specific periodontal pathogens are responsible for the progressive form of the disease. However, some individuals harbor these specific microorganisms, but do not appear to show evidence of disease progression. Patient susceptibility is of utmost importance to the outcome of periodontal disease, and although periodontal bacteria are the major etiological agents, the host immune response to these bacteria is of fundamental importance (2).

Histological studies support the concept that the immune system responds to plaque microorganisms. The infiltrate in the periodontal lesion consists of lymphocytes and macrophages; whereas T lymphocytes predominate in the stable lesion, the proportion of B cells and plasma cells is increased in the progressive lesion (1, 2). Functional assays using peripheral blood lymphocytes have contributed to the understanding of the disease process by highlighting a role for T cells. Most work on immunoregulation of chronic inflammatory periodontal disease has therefore focused on T cells (3). Studies have shown a depressed CD4:CD8 ratio in cells extracted from adult periodontitis lesions compared with peripheral blood and healthy tissue or tissue with gingivitis (4, 5), and T cells extracted from diseased periodontal tissues have a reduced ability to respond in an autologous mixed lymphocyte reaction (6), suggesting a suppression of cell-mediated responses. Seymour et al. (7) demonstrated a lack of interleukin 2 (IL-2) production by unstimulated T cells extracted from adult periodontitis tissue and suggested this as a reason for the failure of these cells to undergo spontaneous proliferation (4).

Although patient susceptibility is of major importance in determining the outcome of periodontal disease, the problems in detecting susceptible individuals have not been solved. There is a great deal of variation in the microbial composition between individuals and also from site to site in the same individual, as well as variation with respect to the frequency and rate of progression of periodontal disease (2). The study of cytokines in periodontal disease lesions may throw some light on this problem and may also suggest future lines of therapy.

Cytokines are recognized as being vital in the immunopathology of an ever-increasing number of diseases, and the production of 'appropriate' cytokines is essential for the development of protective immunity. If 'inappropriate' cytokines are elicited, destructive or progressive disease can result (8). Just how the immune system selects the right response to a particular pathogen is not clear (9). However, determination of the features of both the host and pathogen that direct how and where the organism is presented to cytokine-producing cells is necessary if we are to understand the pathogenesis of not only periodontal but all infectious diseases (8).

Cytokines are cell regulators that have a major influence

on the production and activation of different effector cells. T cells and macrophages are a major source, although they are produced by a wide range of cells that play important roles in many physiological responses. Cytokines are low-molecular-weight proteins involved in the initiation and effector stages of immunity and inflammation, in which they regulate the amplitude and duration of the response. They are usually produced transiently, are extremely potent, generally acting at picomolar concentrations and interact with specific cell surface receptors, which are usually expressed in relatively low numbers (10). Some cytokines are produced by a restricted type of cell, such as IL-2 produced by T cells, whereas others, including IL-1 and IL-6, are produced by many very different cell types. Target cells may also be restricted or very diverse (11). Many cytokines are pleiotropic, having multiple activities on different target cells and or overlapping cell regulatory actions (11). 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. Cytokines interact in a network: first by inducing each other; second by transmodulating cell surface receptors; and third by synergistic, additive, or antagonistic interactions on cell function (10).

The majority of immune responses occur locally rather than systemically within a small area of tissue and often between two cells that are conjugated to one another (12). Since the discovery of IL-2 in 1976, more than 20 cytokines have been characterized that are essential for many of the proliferative and differentiative functions of immune cells (12). There thus appears to be a very complex network of interactions within the immune system. Mosmann (12) believes that this complexity is essential for overcoming the various defense strategies of microorganisms. As microorganisms evolve more rapidly than their mammalian hosts, a single immune mechanism could not cope with every new product synthesized by an infectious agent. Multiple regulatory mechanisms may therefore be a defense against pathogen interference and hence could be essential in the preservation of homeostasis.

Interleukin-1 (IL-1)

It is now generally agreed that IL-1 is the major mediator of tissue destruction in periodontal disease (13). What is not agreed, however, is the source of this IL-1. Most workers believe that macrophages are the primary source, but few studies have actually been reported on macrophages in periodontal disease. While *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* have been shown to activate monocytes and macrophages and stimulate the secretion of proinflammatory and tissue destructive mediators (14), a recent report has shown that there is no increase in macrophage numbers and little evidence of macrophage activation in advanced periodontitis compared with minimally inflamed tissues (15). This is supported by reports demonstrating negative effects by

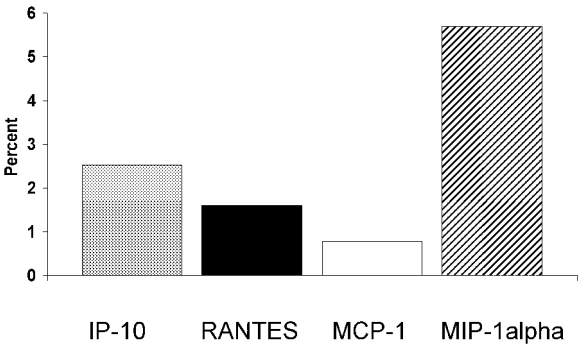

Fig. 1. Chemokine expression in periodontitis tissues.

periodontal pathogens on monocytes/macrophages. *P. gingivalis* has, for example, been shown to affect macrophage migration and activation by inhibiting the production of one of the major monocyte/macrophage chemokines, monocyte chemoattractant protein-1 (MCP-1) (16). More recently, using immunoperoxidase, Gemmell et al. (17) have shown that numbers of MCP-1-positive cells were notably fewer compared with other chemokine-positive cells, with 30 out of 47 specimens demonstrating less than 1% positive cells. This reduction in MCP-1-positive cells is most obvious in the more severely inflamed tissues (Fig. 1).

As noted above, Chapple et al. (15) used a number of markers of key macrophage function to study these populations in advanced periodontal diseased tissues compared with minimally inflamed tissues. They clearly demonstrated that the numbers of macrophages did not increase in advanced lesions and there was little evidence of their activation despite the extensive tissue destruction (15). In another study (18), intramuscular injections of *P. gingivalis* LPS in mice resulted in a reduction or absence of MCP-1 4 h later and few cells in the *P. gingivalis* LPS-injected animals expressed MCP-1 mRNA. LPS injection has also been shown to down-regulate the expression of CCR2, a specific receptor for MCP-1 on peripheral blood cells, resulting in the blocking of macrophage infiltration into the peritoneal cavity of mice in response to thioglycollate (19).

Gemmell et al. (17) also demonstrated that endothelial cells expressed MCP-1; however, positive capillaries were located only in areas devoid of infiltrating cells and the numbers of tissue samples exhibiting positive staining decreased with increasing size of infiltrate. These results suggest that in the early stages of gingival inflammation, monocytes/macrophages may readily migrate into the tissues via MCP-1-positive endothelial cells; with increasing inflammation, however, expression of MCP-1 is abrogated leading to a reduction of this cell type in the tissues. This suggests that macrophages may have protective effects in the stable lesion which are abrogated in the advanced destructive lesion.

The high numbers of MIP-1α-positive cells seen by Gemmell et al. (17) are interesting. These workers noted

that the percent MIP-1α-positive cells were significantly higher with increasing proportions of CD8 cells and B cells, which may in part explain the increase in these cell types in the progressive lesion. Taken together these results cast doubt on the role of macrophages as the primary source of IL-1 in advanced periodontitis. In addition, Gemmell & Seymour (20) have shown that *P. gingivalis* induces IL-1 production by B cells rather than monocytes in periodontitis patients. This suggests that lymphocytes (B cells) may be a major source of IL-1 in periodontitis.

As with all inflammatory reactions, it is the balance of cytokines that leads to the ultimate outcomes. In this content, a number of cytokines such as IL-10 and IL-11 are known to down-regulate IL-1 production. Recently, Martuscelli et al. (21) have shown that subcutaneous injection of recombinant IL-11 significantly reduced periodontal attachment loss in a beagle dog, ligature induced, model. It could be speculated that periodontal tissue destruction is a result of the unregulated production of IL-1 by B cells in the tissues.

Immunoregulation in periodontal disease: the Th1 and Th2 paradigm

Over the years, a number of theories have been postulated to explain the progression of gingivitis to periodontitis. In the past decade, most of these theories have revolved around the Th1/Th2 paradigm. Immunohistological studies have clearly established that a T cell/macrophage lesion identical to a delayed hypersensitivity reaction (22) occurs within 4 to 8 days of plaque accumulation in an experimental gingivitis study (23).

This is synonymous with the early lesion of Page & Schroeder (24) and with the putative stable lesion (25). The expression of HLA-DR and DQ by these cells indicates that they are activated, but the lack of CD25 expression would indicate that they are not proliferating locally within the tissues (26). The striking similarities between this early/stable periodontal lesion and delayed type hypersensitivity prompted the suggestion that cells with a Th1 cytokine profile are the major mediators. Such a concept is consistent with the proposal that a strong innate immune response leads to the production of IL-12, which in turn leads to this Th1 response. The production of IFN-γ then enhances the phagocytic activity of both neutrophils and macrophages and hence containment of the infection. However, the stable lesion persists due to the continual formation of the plaque biofilm (27).

The dominance of B cells/plasma cells in the advanced/progressive lesion would suggest a role for Th2 cells. Clearly, if the innate response is poor, low levels of IL-12 would be produced and a poor Th1 response may occur which may not then contain the infection. Polyclonal B cell activation and the subsequent production of IL-4 would encourage a Th2 response, further B cell activation, and antibody production. If these antibodies are protective and clear the infection, the disease will not progress, but if on the other hand they are non-protective, the lesion will persist and continued B cell activation may result in the unregulated production of large amounts of IL-1 and subsequent tissue destruction (20, 27, 28).

In order to test this hypothesis, a number of studies have attempted to delineate the Th1/Th2 profile in periodontal disease. However, results are difficult to interpret due to differences in material examined and methodologies used. Cytokines have been studied in cells in situ, cells extracted from gingival tissues, peripheral blood mononuclear cells, T cell lines and clones as well as purified cell populations. A variety of techniques have also been used, including flow cytometry, enzyme linked immunosorbent assay (ELISA), in situ hybridization, and reverse transcriptase polymerase chain reaction (RT-PCR). Additionally, bacterial components, including sonicates, heat and formalin-killed cells, outer membrane components, and purified antigens have all been used to stimulate cells in vitro, making comparisons of cytokine profiles difficult (14). It is likely that different T cell subsets predominate at different phases of disease and the inability to determine disease activity clinically is a major limitation in all these studies.

A number of studies have reported decreased Th1 responses in periodontal disease. Pilon et al. (29) demonstrated lower levels of IL-2 in the gingival crevicular fluid of periodontitis sites compared with healthy sites, and Fujihashi et al. (30) have shown that gingival mononuclear cells from adult periodontitis patients produce IL-4 and IL-5 but not IL-2. Peripheral blood mononuclear cells from periodontitis patients stimulated with mitogens resulted in reduced IFN-γ secretion and mRNA expression for IFN-γ and IL-2. At the same time, significantly higher levels of IL-5 and GM-CSF were observed (31).

Increased Th2 responses in periodontitis have also been reported. Memory T cells from the peripheral blood of adult periodontitis patients with high anti-*P. gingivalis* titres, stimulated in vitro with *P. gingivalis*, have been shown to produce higher amounts of IL-4 than cells from healthy subjects (32). In this study, no IL-4-producing memory T cells were detected in healthy gingival tissues and a larger proportion of peripheral blood memory T cells from patients in which high frequencies of IL-4 producing cells were identified in the lesion, produced IL-4 following stimulation with antigen. Yamazaki et al. (33) demonstrated an increased percent IL-4 positive cells proportional to an increasing B cell/T cell ratio. IL-4 was the prominent cytokine in periodontally affected tissues compared with IL-2, IFN-γ and IL-6. Another study suggested a role for IL-4 and Th2 responses in periodontitis lesions by the demonstration of concentrations of IgG_4 many times higher in sites of active periodontitis than in serum as well as significantly elevated concentrations compared with stable lesions (34). A bias towards Th2-type cytokines in periodontal disease progression was also indicated when cytokine analysis of cells in inflamed gingiva by in situ hybridization showed that the density of cells expressing IL-1-α, IL-4 and IL-5 mRNA was higher

in periodontitis than in gingivitis (35). Further, cell dot blot analysis of cytokine-producing gingival mononuclear cells showed that a higher percent of non-stimulated periodontal disease cells were IL-4 positive (36). Analysis of IL-2/IL-4 ratios revealed significantly lower ratios for cells derived from periodontitis tissues compared with cells from gingivitis tissues. An immunohistochemical study demonstrated a significantly higher level of IL-4-producing cells in periodontal lesions in comparison with gingivitis tissues, and although this was also the case for IL-6 producing cells, the results were not significant (37). In this study, Th2-type cells were shown to accumulate in periodontitis. In another study, peripheral blood mononuclear cells isolated from *P. gingivalis*-positive gingivitis and periodontitis subjects were stimulated with *P. gingivalis* outer membrane antigens. IL-4 positive T cells predominated over IFN-γ positive and IL-10 positive cells (20). Taken together, these data support the hypothesis that Th1 cells are associated with the stable lesion and a Th2 response with disease progression.

In contrast to these studies, Ebersole & Taubman (38) found that IFN-γ message was prominently expressed by diseased gingival tissue cells. Cytokine profiles of cells extracted from 6 patients were consistent with Th1 cells in that they were IL-2 and IFN-γ positive, but negative for IL-4 and IL-5. A further sample had message for IL-2 and IL-5, consistent with Th0 cells. The inability to detect IL-4 mRNA in these experiments could have been due to its very short half-life rather than its absence from the tissues. As with other studies, disease activity was not clearly defined, such that again interpretation is difficult. Another study comparing the local and systemic responses in periodontitis patients with so-called terminal dentition periodontitis demonstrated reduced Th2 responses. Levels of PGE_2, IL-1 beta, and IL-2 in the gingival crevicular fluid samples were highest followed by lower levels of TNF-alpha and IFN-gamma and even lower levels of IL-4 and IL-6 (39). Cytokine mRNA expression of Th1 and Th2 cytokines was present in isolated gingival mononuclear cells with low levels of IL-4 and IL-12. While it was concluded that Th1 cytokine levels dominated over the Th2 response in the gingival crevicular samples, because different concentrations of different cytokines result in different biological activity, comparing different levels of cytokines in the same patient is inappropriate. It would have been of greater value had the levels of the same cytokines been compared in patients from different disease groups. Interpretation of this study is therefore difficult.

Other studies have suggested the involvement of Th0 cells in periodontal disease. Fujihashi et al. (40) extract mRNA for IFN-γ, IL-6, and IL-13, but not for IL-2, IL-4, or IL-5 in CD4-positive T cells from periodontal disease lesions. Takeichi et al. (41) showed that IFN-γ and IL-1-β mRNA were expressed by some gingival cells on extraction, indicative of Type 1 cells, and upon stimulation IL-6 transcripts were also expressed but no IL-2 or IL-2 receptor mRNA could be detected. Yet another study found no skewing of cytokines towards a Th1 or Th2 profile in diseased or healthy tissues, although there was a significantly higher expression of IL-6 and IFN-α mRNA in diseased tissue (42). Fujihashi et al. (43) isolated CD4 cells from inflamed gingival tissues and demonstrated two profiles both of which were positive for IFN-γ, IL-6, and IL-13 mRNA, while one group and not the other was also positive for IL-10. Nakajima et al. (44) demonstrated that stimulation of peripheral blood mononuclear cells from individuals with periodontitis and gingivitis with *P. gingivalis* resulted in upregulation of messenger RNA for IFN-γ and IL-13, while IL-4 and IL-10 were downregulated irrespective of disease status or the presence of *P. gingivalis* in plaque samples. Yamazaki et al. (45) have shown that the mean expression of IFN-γ mRNA was higher in the peripheral circulation than the gingival tissues, while that of IL-10 mRNA was higher in the gingival tissues. However, only 7/16 samples demonstrated a high expression of IL-10, the other samples showing equivalent levels in blood and tissues. IL-12 mRNA was similarly expressed to a higher extent in 6/16 samples, the other samples showing no differences between gingiva and peripheral blood. IL-4 mRNA was weak but detectable in 3 samples. Because of the lack of clinical markers for disease activity, interpretation of these studies is difficult. It could be for example that the subjects with high IL-10 levels were in a quiescent phase and those with high IL-12 may also be stable. The reverse could also be true, those with high IL-12 could have a progressive Th1 response.

Studies on T cell lines and clones have also demonstrated conflicting results. Flow cytometry and RT-PCR have been used to show that CD4 and CD8 cells in *P. gingivalis*-specific lines and clones derived from a *P. gingivalis*-positive gingivitis subject and a *P. gingivalis*-positive periodontitis patient produced IL-4, IL-10 and IFN-γ (46). However, in a further study, a number of lines established from *P. gingivalis*-positive gingivitis and periodontitis subjects demonstrated highly variable profiles, although the mean results showed that a high percent of both CD4 and CD8 cells were positive for IFN-γ with lower percentages of IL-4 positive and IFN-γ-positive cells. Lines established from the periodontitis subjects demonstrated more variation than lines from the gingivitis individuals, such that IL-4-positive and/or IL-10-positive T cells predominated over IFN-γ-positive cells in a greater number of lines (47). Wassenaar et al. (48) established T cells clones from the gingival tissues of 4 patients with chronic periodontitis. Eighty percent of the CD4 clones had Th2 phenotypes producing high levels of IL-4 and low levels of IFN-γ.

While it is difficult to interpret all these studies, the pattern seems to be emerging that, within the tissue, gingivitis seems to be associated with a Th1 response, while periodontitis is associated with a Th2 response. This is consistent with the histology of these two diseases. In peripheral blood, however, no clear pattern is emerging. The reasons for this remain obscure.

Emanating from studies of cytokines in periodontal disease is the concept that IL-10 may be of fundamental importance in the control of periodontal disease progression (49). Yamamoto et al. (50) were able to show 2 distinct cytokine patterns in cells isolated from periodontitis tissues. One pattern showed the presence of IFN-γ, IL-6, IL-10, and IL-13 mRNA, while the other was similar except that it lacked IL-10 mRNA. It may be that those lesions producing high levels of IL-10 remain stable, while those with low levels progress. However, caution must be exercised in interpreting cytokine data. The in-built redundancy within cytokine networks ensures that if one cytokine is absent another with similar activity may take its place. In this context IL-6 and TNF-α have many of the functions of IL-1, IL-13 can replace IL-4 and in many respects IL-11 may replace IL-10. As yet, the cytokine networks in periodontal disease have not been fully elucidated and caution must be taken in interpreting the data thus far. This is further reinforced with the finding that some cytokines can have apparently opposing functions. The recently described IL-18, for example, can act synergistically with IL-12 to promote a Th1 response. At the same time, it has been shown to enhance Th2 cytokine production (51). IL-18 has also been shown to inhibit osteoclast maturation, hence reducing bone resorption. In addition, it suppresses COX (cyclooxygenase) expression through IFN-γ production but at the same time may increase COX 2 expression (51). As yet, IL-18 has not been described in periodontal tissues, but it is clear that further studies to elucidate the interplay between IL-18/IL-12/IL-15 within the periodontal tissues are necessary.

Role of antigen-presenting cells (APC)

If the hypothesis that Th1 cells are associated with the stable lesion and Th2 cells are associated with the progressive lesion is true, it would follow that therapies aimed at enhancing a Th1 profile may have some role in the treatment of advanced or even refractory periodontal disease. In this context, therefore, control of the Th1/Th2 profile would be fundamental. Equally, if the reverse hypothesis is true, treatments aimed at enhancing a Th2 response and subsequent specific antibody production would be desirable. In either case the ability to modulate the Th1/Th2 profile may have significant therapeutic implications.

The production of Th1 cytokines is fundamentally dependent upon IL-12. IL-12 was originally described as a factor promoting natural killer and cytotoxic T cell activity. It is produced by monocytes, macrophages, dendritic cells, and polymorphonuclear neutrophils. B cells produce very low levels. In contrast, Th2 cytokine production is dependent upon the presence of IL-4. IL-4 in turn is produced by Th2 T cells, mast cells, and transformed B cells.

It was originally thought that upon activation with Ag, Th0 cells would differentiate into either Th1 or Th2 cells. However, Kelso (52) has proposed that Th1 and Th2 cells form a continuum such that depending upon the local environmental conditions a particular clone can secrete either Th1 or Th2 cytokines or indeed both. In other words the Th1 or Th2 profile is not fixed. Gemmell et al. (47) showed that when peripheral blood mononuclear cells were used to present *P. gingivalis* antigens to *P. gingivalis*-specific T cell lines, highly varied cytokines profiles resulted. This is possibly due to a number of cells, including dendritic cells, monocytes and B cells all acting as antigen-presenting cells within the peripheral blood. Using the same cell lines, these workers have now been able to show that when *P. gingivalis* antigens are presented by autologous EBV-transformed B cells (LCL), the cytokine profiles are predominantly IL-4 positive, with lower numbers of IFN-γ-positive T cells and very few IL-10-positive T cells and that these profiles are constant for every T cell line (Fig. 2).

These results, while not definitively proving the contention that Th0, Th1, and Th2 cells all form a continuum (52), do show that the nature of the APC is fundamental in determining whether or not a Th1 or Th2 cytokine profile develops. In this context it may be possible to modulate the Th1/Th2 profile therapeutically so as to induce a protective response in an otherwise susceptible individual.

Dendritic cell (DC) vaccines are currently being used as cancer immunotherapeutic agents (53). In these studies, autologous DCs are being pulsed with cancer antigens before being injected back into the patient in an attempt to stimulate IFN-γ secreting CD8 cytotoxic T cells. It may be that, in the future, autologous DCs pulsed with specific periodontopathic bacterial antigens may be a useful therapeutic modality.

Recently, Choi et al. (54) have shown that T cell clones derived from mice immunized with *Fusobacterium nucleatum* followed by *P. gingivalis* demonstrated a Th2 profile, while those derived from mice immunized with *P. gingivalis* alone all demonstrated a Th1 profile. While these results remain to be confirmed they support the concept that complexes

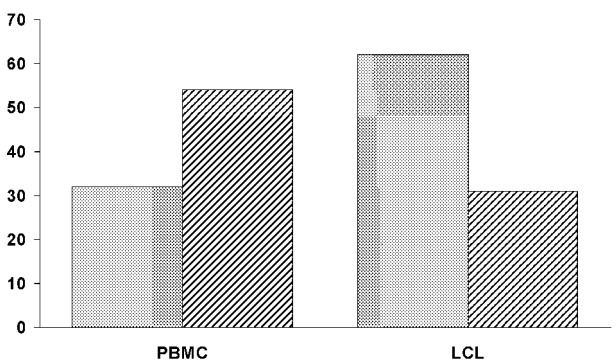

Fig. 2. IL-4 (▒) and IFN-γ (▨) *Pg* specific CD4 T cells using peripheral blood mononuclear cells (PBMC) and autologous EBV-transformed B cells (LCL) as antigen presenting cells (APC).

of organisms are necessary and the presence of a polyclonal B cell activator such as *F. nucleatum* may be necessary to promote a Th2 response. If so, these findings could point therapeutic modalities back to modifying the composition of plaque.

References

1. Seymour GJ. Possible mechanisms involved in the immunoregulation of chronic inflammatory periodontal disease. J Dent Res 1987;66:2–9.
2. Seymour GJ. Importance of the host response in the periodontium. J Clin Periodontol 1991;18:421–6.
3. Seymour GJ, Gemmel E, Reinhardt RA, Eastcott J, Taubman MA. Immunopathogenesis of chronic inflammatory periodontal disease: cellular and molecular mechanisms. J Periodontal Res 1993;28:478–86.
4. Cole KC, Seymour GJ, Powell RN. The autologous mixed lymphocyte reactions (AMLR) using periodontal lymphocytes. J Dent Res 1986;65:473.
5. Taubman MA, Stoufi ED, Ebersole JL, Smith DJ. Phenotypic studies of cells from periodontal disease tissue. J Periodontal Res 1984;19:587–90.
6. Cole KC, Seymour GJ, Powell RN. Phenotypic and functional analysis of T cells extracted from chronically inflamed human periodontal tissues. J Periodontol 1987;58:569–73.
7. Seymour GJ, Cole KL, Powell RN, Lewins E, Cripps AW, Clancy RL. Interleukin-2 production and bone resorption activity by unstimulated lymphocytes extracted from chronically inflamed human periodontal tissues. Arch Oral Biol 1985;30: 481–4.
8. Kelso A. Cytokines in infectious disease. Aust Microbiol 1990; 11:372–6.
9. Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol Today 1996;17:138–46.
10. Balkwill FR, Burke F. The cytokine network. Immunol Today 1989;9:299–304.
11. Hamblin AS. In: Male D, editor. Cytokines and cytokine receptors. Oxford: Oxford University Press; 1993.
12. Mosmann TR. Cytokines: is there biological meaning? Curr Opin Immunol 1991;3:311–4.
13. Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. Advances in the pathogenesis of periodontitis: summary of developments, clinical implication and future directions. Periodontology 2000;14:216–48.
14. Zadeh HH, Nicols FC, Miyasaki KT. The role of the cell-mediated immune response to *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in periodontitis. Periodontol 2000;20: 239–88.
15. Chapple CC, Srivastava M, Hunter N. Failure of macrophage activation in destructive periodontal disease. J Pathol 1998; 186:281–6.
16. Gemmell E, Grieco DA, Seymour GJ. Chemokine expression in *Porphyromonas gingivalis*-specific T-cell lines. Oral Microbiol Immunol 2000;15:166–71.
17. Gemmell E, Carter CL, Seymour GJ. Chemokines in human periodontal disease tissues. Clin Exp Immunol 2001. In press.
18. Reife RA, Shapiro RA, Bamber BA, Berry KK, Mick GE, Darveau RP. *Porphyromonas gingivalis* lipopolysaccharide is poorly recognized by molecular components of innate host defense in a mouse model of early inflammation. Infect Immun 1995;63: 4686–94.
19. Zhou Y, Yang Y, Warr G, Bravo R. LPS down-regulates the expression of chemokine receptor CCR2 in mice and abolishes macrophage infiltration in acute inflammation. J Leukoc Biol 1999;65:265–9.
20. Gemmell E, Seymour GJ. Cytokine profiles of cells extracted from humans with periodontal diseases. J Dent Res 1998;77:16–26.
21. Martuscelli G, Fiorellini JP, Crohin CC, Howell TH. The effect of interleukin-11 on the progression of ligature-induced periodontal disease in the beagle dog. J Periodontol 2000;71:573–8.
22. Poulter LW, Seymour GJ, Duke O, Janossy G, Panayi G. Immunohistological analysis of delayed-type hypersensitivity in man. Cell Immunol 1982;74:358–69.
23. Seymour GJ, Powell RN, Aitken JF. Experimental gingivitis in humans. A clinical and histologic investigation. J Periodontol 1983;54:522–8.
24. Page RC, Schroeder HE. Pathogenesis of inflammatory periodontal disease. A summary of current work. Lab Invest 1976;34: 235–249.
25. Seymour GJ, Powell, RN, Davies WI. Conversion of a stable T-cell lesion to a progressive B-cell lesion in the pathogenesis of chronic inflammatory periodontal disease: an hypothesis. J Clin Periodontol 1979;6:267–77.
26. Seymour GJ, Gemmell E, Walsh LJ, Powell RN. Immunohistological analysis of experimental gingivitis in humans. Clin Exp Immunol 1988;71:132–7.
27. Gemmell E, Seymour GJ. Modulation of immune responses to periodontal bacteria. Curr Opin Periodont 1994;94:28–38.
28. Seymour GJ, Gemmell E, Reinhardt RA, Eastcott J, Taubman MA. Immunopathogenesis of chronic inflammatory periodontal disease: cellular and molecular mechanisms. J Periodontal Res 1993;28:478–86.
29. Pilon M, Williams-Miller C, Cox DS. Interleukin-2 levels in gingival crevicular fluid in periodontitis. J Dent Res 1991;70:550.
30. Fujihashi K, Kono Y, Yamamoto M, McGhee JR, Beagley K, Aicher WK, et al. Interleukin production by gingival mononuclear cells isolated from adult periodontitis patients. J Dent Res 1991;70:550.
31. Sigusch B, Klinger G, Glockmann E, Simon Hu. Early-onset and adult periodontitis associated with abnormal cytokine production by activated T lymphocytes. J Periodontol 1998;69:1098–104.
32. Aoyagi T, Sugawara-Aoyagi M, Yamazaki K, Hara K. Interleukin 4 (IL-4) and IL-6-producing memory T-cells in peripheral blood and gingival tissues in periodontitis patients with high serum antibody titers to *Porphyromonas gingivalis*. Oral Microbiol Immunol 1995;10:304–10.
33. Yamazaki K, Nakajima T, Aoyagi T, Hara K. Immunohistological analysis of memory T lymphocytes and activated B lymphocytes in tissues with periodontal disease. J Periodontal Res 1994;28:324–34.
34. Reinhardt RA, McDonald TL, Bolton RW, Dubois LM, Kaldahl WB. IgG subclasses in gingival crevicular fluid from active versus stable periodontal sites. J Periodontol 1989;60;44–50.
35. Tokoro Y, Matsuki Y, Yamamoto T, Suzuki T, Hara K. Relevance of local Th2-type cytokine mRNA expression in immunocompetent infiltrates in inflamed gingival tissue to periodontal diseases. Clin Exp Immunol 1997;107:166–74.
36. Manhart SS, Reinhardt RA, Payne JB, Seymour GJ, Gemmell E, Dyer JK, et al. Gingival cell IL-2 and IL-4 in early-onset periodontitis. J Periodontol 1994;65:807–13.
37. Yamazaki K, Nakajima T, Gemmell E, Polak B, Seymour GJ, Hara K. IL-4- and IL-6-producing cells in human periodontal disease tissue. J Oral Pathol Med 1994;23:347–53.
38. Ebersole JL, Taubman MA. The protective nature of host responses in periodontal diseases. Periodontology 2000 1994;5: 112–41.
39. Salvi GE, Brown CE, Fujihashi K, Kiyono H, Smith FW, Beck JD, et al. Inflammatory mediators of the terminal dentition in adult and early onset periodontitis. J Periodontal Res 1998;33: 212–25.
40. Fujihashi K, Yamamoto M, McGhee JR, Kiyono H. Type 1/ Type 2 cytokine production by CD4+ T cells in adult periodontitis. J Dent Res 1994;73:204. Abstract 818.
41. Takeichi O, Taubman MA, Haber J, Smith DJ, Moro I.

Cytokine profiles of CD4 and CD8 T cells isolated from adult periodontitis gingivae. J Dent Res 1994;73:205.
42. Prabhu A, Michalowicz Mathur A. Detection of local and systemic cytokines in adult periodontitis. J Periodontol 1996;67: 515–22.
43. Fujihashi K, Yamamoto M, Hiroi T, Bamberg TV, McGhee JR, Kiyono H. Selected Th1 and Th2 cytokine mRNA expression by CD4(+) T cells isolated from inflamed human gingival tissues. Clin Exp Immunol 1996;103:422–8.
44. Nakajima T, Yamazaki K, Cullinan MP, Gemmell E, Seymour GJ. T-cell antigen specificity in humans following stimulation with *Porphyromonas gingivalis*. Arch Oral Biol 1999;44:1045–53.
45. Yamazaki K, Nakajima T, Kubota Y, Gemmell E, Seymour GJ, Hara K. Cytokine messenger RNA expression in chronic inflammatory periodontal disease. Oral Microbiol Immunol 1997;12:281–7.
46. Gemmell E, Kjeldsen M, Yamazaki K, Nakajima T, Aldred MJ, Seymour GJ. Cytokine profiles of *Porphyromonas gingivalis*-reactive T lymphocyte line and clones derived from *P. gingivalis*-infected subjects. Oral Dis 1995;1:139–46.
47. Gemmell E, Grieco DA, Cullinan MP, Westerman B, Seymour GJ. The proportion of interleukin-4, interferon-10-positive cells in *Porphyromonas gingivalis*-specific T-cell lines established from *P. gingivalis*-positive subjects. Oral Microbiol Immunol 1999;14: 267–74.
48. Wassenaar A, Reinhardus C, Thepen T, Abraham Inpijn L, Kievits F. Cloning, characterization, and antigen specificity of T-lymphocyte subsets extracted from gingival tissue of chronic adult periodontitis patients. Infect Immun 1995;63:2147–53.
49. Gemmell E, Marshall RI, Seymour GJ. Cytokines and prostaglandins in immune homeostasis and tissue destruction in periodontal disease. Periodontol 2000 1997;14:112–43.
50. Yamamoto M, Fujihashi K, Hiroi T, McGhee JR, Van Dyke TE, Kiyono H. Molecular and cellular mechanisms for periodontal diseases: role of Th1 and Th2 type cytokines in induction of mucosal inflammation. J Periodontal Res 1997;32:115–9.
51. McInnes IB, Gracie JA, Leung BP, Wei X-Q, Liew FY. Interleukin-18: a pleiotropic participant in chronic inflammation. Immunol Today 2000;21:312–4.
52. Kelso A. Th1 and Th2 subsets: paradigms lost? Immunol Today 1995;16:374–9.
53. Hart DN. Dendritic cells: unique leukocyte populations which control the primary immune response. Blood 1997;90:3245–87.
54. Choi JI, Borrello MA, Smith ES, Zauderer M. Polarization of *Porphyromonas gingivalis*-specific helper T-cell subsets by prior immunization with *Fusobacterium nucleatum*. Oral Microbiol Immunol 2000;15:181–7.