J Clin Periodontol 2011; 38 (Suppl. 11): 44–48 doi: 10.1111/j.1600-051X.2010.01682.x

Journal of Clinical Periodontology

Host-response: understanding the cellular and molecular mechanisms of host-microbial interactions – Consensus of the Seventh European Workshop on Periodontology

Denis F. Kinane, Philip M. Preshaw and Bruno G. Loos, on Behalf of Working Group 2 of the Seventh European Workshop on Periodontology (in alphabetical order)

Abstract

Background: Major challenges in periodontology include understanding the pathophysiology, the interplay between various components of the host response, parallels with other diseases and identifying biomarkers of the disease.

Objectives: Four reviews were compiled with the aim of better understanding: (1) the role of polymorphic nuclear leucocytes (PMNs), i.e. neutrophils; (2) the function of cytokine networks in the host response; (3) whether parallels exist with rheumatoid arthritis (RA); and (4) whether useful biomarkers currently exist to help in the management of periodontal disease.

Material and Methods: Based on the focused questions, electronic and manual searches were conducted for human, animal and cellular studies on the above topics.

Results: Papers fulfilling the inclusion criteria were selected and reviews were written and reviewed and corrected before the academy meeting to produce consensus statements.

Conclusion: The following consensus statements were produced. PMNs are important in the pathophysiology of periodontal disease but there is limited evidence on their much quoted destructive potential. Cytokine networks are enormously complex and we are really at the beginning of understanding their role in the disease process. RA has both similarities and marked differences to periodontal disease although the existing utilization of anti-cytokine therapies and other molecules in its treatment may have importance in periodontal disease therapy. Biomarkers for periodontal disease have yet to be completely defined but the ratio of receptor activator of NF-κB ligand to osteoprotegerin appears to be a biomarker test with utility for detecting bone destruction.

Working Group 2: Nurcan Buduneli, Paul Cooper, Xavi Costa Berenguer, Francesco D'Aiuto, Mauro Donati, Steven Engebretson, Marta Escribano, Anders Gustafsson, Francis J. Hughes, Sören Jepsen, F. Y. (Eddy) Liew, Phoebus Madianos, Jörg Meyle, Gregory J. Seymour, Lior Shapira, Leonardo Trombelli

Conflict of interest and source of funding statement

This workshop has been financially supported by an unrestricted educational grant from Colgate. The sponsor had no impact on the programme or on the deliberations of the European Workshop. Group participants declared that they had no conflict of interests.

How has neutrophil research improved our understanding of periodontal pathogenesis? (Nussbaum & Shapira 2011)

1. Does periodontal disease occur due to polymorphic nuclear leucocytes (PMNs) number or functional abnormalities/deficiencies?

Experiments of nature (neutropenia, etc.) indicate that sufficient PMN counts are critical for periodontal health. In addition, in experiments of nature where PMN function is abrogated such as in LAD, severe generalized periodontal destruction ensues and typically results in early loss of the dentition. The severe generalized periodontal destruction and the disease process in these conditions may be quite different to that seen in chronic periodontitis (CP) and aggressive periodontitis (AgP).

Not all deficiencies in PMNs lead to periodontal destruction, e.g. chronic granulomatous disease (CGD). Subjects with PMN defects in oxidative burst such as CGD, have been shown not to be more prone to periodontitis. Interestingly, ''oxidase null mice'' were significantly more resistant to infection by *Porphyromonas gingivalis*.

2. Is there subject variation in PMN function and are these variations relevant to disease? Can we detect these variations and how?

The evidence that specific variations in PMN function contribute to periodontitis in otherwise healthy subjects is low. It is possible that in some cases genetic polymorphisms related to PMN function may help subsets of periodontal pathogens evade the PMN response or may lead to PMN hyperactivity, however this most likely only contributes to disease pathogenesis in a minority of CP or AgP cases.

Multiple studies over the years have reported conflicting results and thus provide inconclusive evidence that there are functional variations that are relevant to periodontitis.

3. What is the role of PMNs in periodontal tissue destruction?

Although the principal role of PMNs is protective, PMNs can release a wide variety of factors that will damage host tissues including reactive oxygen species, collagenases and other proteases. Clearly the number of PMNs in the inflammatory infiltrate would suggest that they have importance in tissue destruction. Although the data from in vitro and animal experiments supports an important role of PMNs in tissue destruction, it is difficult to determine the relevance of any single cell type to the natural history of the disease. Anti-apoptotic activity (by both mitochondrial and cytoplasmic pathways) in PMNs may encourage the persistence of PMNs and further contribute to periodontal tissue damage. Further evidence to support this hypothesis comes from experiments on molecules that drive the resolution of inflammation through limiting PMN activity and promoting apoptosis. PMNs in common with other cells in the infiltrate, express molecules including cytokines and receptor activator of NF-κb ligand (RANKL) that are implicated in bone resorption.

4. Is there any evidence that microbial components might kill or alter function of PMNs?

There is in vitro evidence that components of the oral biofilm, including *Aggregatibacter actinomycetemcomitans* leukotoxin (which can kill PMNs), proteases and other microbially derived molecules such as LPS and waste products, can modify PMN functions such as migration or apoptosis.

5. Do patients with periodontitis have hyperactive/reactive PMNs and is this relevant in susceptibility and severity for periodontal disease?

Hyperactivity of PMNs refers to a basal high level of activity and hyper-

Fig. 1. Cytokine networks in periodontal diseases. Schematic to illustrate the multiple interactions between cytokines and cellular functions in periodontal diseases. (1) Resident and infiltrating cells in the periodontium respond to MAMPs signalling via PRRs by production of cytokines as an early step in innate immune responses. Cytokine upregulation is sustained by autocrine and paracrine feedback loops. (Note: question marks (?) indicate more speculative suggestions about the role of specific cytokines in periodontal pathogenesis as known at present.) (2) Upregulated cytokine activity leads to vascular changes, PMN activation and migration, and ultimately, osteoclastogenesis and osteoclast activation. (3) Cytokines produced in innate responses contribute to activation of APCs. These present specific antigens to naive $CD4^+$ T-cells (Th0 cells), which differentiate into $CD4^+$ effector T-cells (e.g. Th1, Th2, Th17, Treg cells) according to the local cytokine milieu (as indicated by the groups of four parallel horizontal grey dotted arrows). For example, Th0 cells differentiate to Th17 cells under the influence of IL-6, IL-21, TGF-β, IL-1β. (APCs are also activated by B-cells, which are themselves activated at a later stage in the cytokine network – indicated by brown dotted arrow at right edge of figure – an example of the complexities of sequential feedback loops that develop.) (4) Th1 and Th2 cells have a relatively stable phenotype, but other T-cell subsets can exhibit functional plasticity under the influence of different cytokine environments (indicated by purple dotted arrows). For example, Th17 cells can develop into Th1 cells under the influence of IL-12, and into Th2 cells under the influence of IL-4. (5) Different T-cell subsets are associated with various cytokine secretion profiles which regulate different aspects of immune responses and contribute to upregulated cytokine activity. For example, Th1 cells secrete IFN-γ (which activates cell-mediated immunity), and Th2 cells regulate antibody-mediated (humoral) immunity through secretion of cytokines IL-4, IL-5 and IL-13. Cytokines produced by different T-cell subsets increase their further secretion in positive feedback loops, and also inhibit development of other T-cell subsets (e.g. IL-4 from Th2 cells inhibits Th1 development, and IFN-γ from Th1 cells inhibits Th2 T-cell subsets). (6) Treg cells secrete TGF-β and IL-10 which have immune suppressive functions. For example, IL-10 suppresses Th1 and Th2 responses, suppresses Mϕ and DC function, and downregulates cytokine production in various cells (Th1 cells, Th2 cells, PMNs, NK cells). (Suppressive effects are indicated by flat-ended green lines.) (7) IL-10 functions as a regulatory mediator, but can also exhibit other activities such as activation of B-cells. The different aspects of IL-10 biology (immunosuppressive *versus* immunostimulatory) likely depend on the local cytokine environment. These dual roles of IL-10 are indicated by the green (inhibitory) line and the black (stimulatory) arrow. (8) The sum total of innate and adaptive effector functions results in an immune-inflammatory response, the precise nature of which will vary from person to person (as indicated by the multiple grey arrows, with some patients being more susceptible to disease than others), and over time within an individual. In this case, the black arrow indicates an individual who has a pro-inflammatory response that leads to connective tissue breakdown and bone resorption. APC, antigen presenting cell; DC, dendritic cell; IFN-γ, interferon-γ; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; MAMP, microbe-associated molecular pattern; Mϕ, cell of monocyte/macrophage lineage; NK, natural killer; PGE_2, prostaglandin E_2; PMN, polymorphonuclear leucocyte; PRR, pattern recognition receptor; RANKL, receptor activator of NF-κB ligand; TGF-β, transforming growth factor-β; Th, T-helper cell; TNF, tumour necrosis factor; Treg, T regulatory cell.

reactive refers to a high level of PMN activity following stimulation.

There are several studies showing that peripheral PMNs from patients with various forms of periodontitis respond more when challenged in vitro compared with PMNs from periodontally healthy individuals. The response could be an increased generation of reactive oxygen species, release of pro-inflammatory cytokines or degranulation, all of which could be relevant to periodontal tissue destruction. This hyperreactivity can to some degree be reversed by successful periodontal therapy.

There is also evidence for an increased basal activity in peripheral PMNs in periodontitis patients which is not affected by periodontal therapy.

It is currently unclear how these hyperactive/reactive PMN states relate to periodontal disease susceptibility.

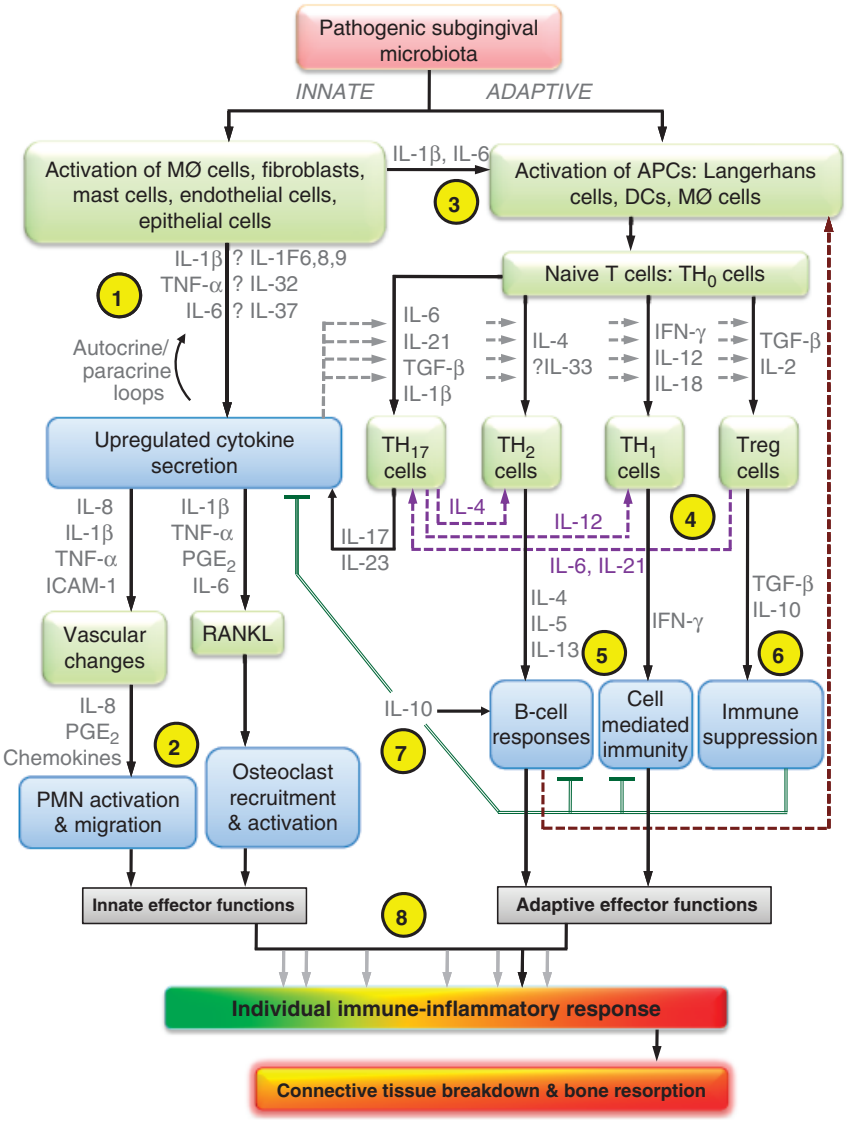
How has research into cytokine interactions and their role in driving immune responses impacted our understanding of periodontitis? (Preshaw & Taylor 2011)

1. What cytokine networks occur in periodontitis?

A cytokine network is defined as a group of cytokines that together modulate and regulate key cellular functions. Cytokine networks in periodontitis are not fully understood. Current knowledge is schematically summarized in Fig. 1. Numerous cytokines are present that play key roles in the pathophysiology of periodontitis. The strongest evidence for cytokines functioning in networks exists for interleukin (IL)-1β, tumour necrosis factor-α (TNF-α), IL-6 and RANKL. More recent data highlight a pro-inflammatory role for IL-10 in periodontitis, in addition to its anti-inflammatory function. In Fig. 1 we have also speculatively included novel cytokines such as IL-32, -33, -37, which are not yet explored in the pathophysiology of periodontitis, but need further characterization within the disease process. Cytokine networks are likely to control T-cell development and plasticity in periodontal lesions (Ford et al. 2010). Variations in these networks may have a major impact on T-cell phenotype and disease expression (Fig. 1), and hence warrant further investigations.

2. Is there individual variation in cytokine networks and does this impact on periodontitis susceptibility, severity and outcome?

Owing to genetic, environmental, epigenetic and microbiota heterogeneity, it is highly likely that there is individual variation in cytokine expression and profiles as outlined in Fig. 1. This could impact on the susceptibility, severity and outcome of the disease. These variations are not yet defined and are a potential area of research. A deep understanding could ultimately lead to personalized therapies.



Host-derived diagnostic markers related to soft tissue destruction and bone degradation in periodontitis (Buduneli & Kinane 2011).

Biomarkers have the potential to provide additional information over standard clinical indices. A periodontal disease biomarker should objectively measure the disease processes involved in the disease pathogenesis and reflect the underlying aetiology in view of ultimately personalized periodontal therapy. In practice there is no such thing as a perfect biomarker, there always is a trade off between specificity and sensitivity. The reason for doing the test will dictate both the choice of biomarker and the need to maximize sensitivity or specificity.

Biomarkers might be useful at the patient as well as at the site level. For site-specific assessment, biomarkers should be collected from GCF. For patient-specific assessment, biomarkers should be sampled from peripheral blood, saliva and pooled GCF from multiple sites.

In reporting the utility of putative biomarkers, the recommendations of the previous EWP workshops on diagnosis and disease definition/progression should be noted (EWP V and VI).

Periodontal disease biomarkers can be grouped as listed below (modified from Wilson et al. 2007):

(1) *Susceptibility*. A biomarker that prospectively identifies individuals or sites at increased risk for periodontal disease.
(2) *Diagnostic*. A biomarker that identifies the presence of periodontal disease.
(3) *Prognostic*. A biomarker that identifies patients or sites most likely to respond to specific interventions.
(4) *Predictive*. A biomarker that predicts future progression of disease.
(5) *Therapeutic*. A biomarker that provides a quantifiable measure of response to periodontal therapy.

All five categories of biomarkers might be useful at the patient as well as at the site level.

1. Are there any promising periodontal disease soluble biomarkers to detect:

(a) Disease susceptibility
We do not currently have reliable biomarkers of susceptibility.

(b) Diagnosis
Most biomarkers reflect inflammation so their ability to discriminate between disease states is limited. There is limited evidence that salivary concentrations of MMP-8 and myeloperoxidase can distinguish between gingivitis and periodontitis. In cross-sectional studies comparing periodontitis and gingivitis patients, differences in the concentration or active states of specific biomarkers can be demonstrated. Because periodontitis differs from gingivitis in terms of bone destruction, biomarkers specific for bone loss such as RANKL/osteoprotegerin ratio (RANKL/OPG) may be more suited to differentiating gingivitis and periodontitis.
Analysis of GCF samples for a range of possible markers including prostaglandin E_2, β glucuronidase, oncostatin M, cathepsin B and K, carboxyterminal telopeptide pyridinoline crosslinks of type 1 collagen (ICPT) may have some diagnostic utility for periodontal disease.
There is currently no reliable biomarker to differentiate CP from AgP.

(c) Prognosis
No existing biomarkers have been proven to have prognostic utility either at the site or patient level.

(d) Predictive
There is limited evidence that certain molecules may be useful biomarkers of future periodontal disease progression.

(e) Therapeutic
Many inflammatory markers have been shown to decrease in response to periodontal treatment.

A number of additional candidate biomarkers are under current investigation but have not yet been shown to have sufficient sensitivity and specificity to use either cross-sectionally for diagnosis or in longitudinal studies to demonstrate, risk, progression or treatment outcome. Host-derived biomarkers might also be combined with information on microbial or genetic factors (for example) to devise composite markers for diagnosis, prognosis or prediction. Combining biomarkers can also add utility but carefully performed prospective studies are needed to prove that they are indeed more useful than single biomarker tests.

In summary, at present a number of biomarkers have been associated with different disease conditions of the periodontal tissues. However, the level of evidence presently available, indicates that currently investigated biomarkers may not provide additional information to chair-side assessment of clinical parameters.

What can the periodontal community learn from the pathophysiology of rheumatoid arthritis (RA) (Culshaw et al. 2011)

1. Are RA and periodontitis similar in terms of aetiology and pathophysiology?

There is a very similar conceptual framework in terms of aetiology and pathophysiology for both diseases (see fig. 1 in Culshaw et al. 2011). For both diseases, environmental factors (e.g. smoking) and genetic factors (e.g. host response genes) play a central role. The essential difference between periodontitis and RA is that RA is an autoimmune disease, while periodontitis is an infectious disease. Nevertheless, in periodontitis there are autoimmune responses against tissue breakdown components (reviewed in Berglundh & Donati 2005).

Periodontitis and RA are similar in that both have a transition phase; for periodontitis, transition from gingivitis to periodontitis is an accepted concept, in RA a pre-articular phase precedes the clinical RA. Further, both diseases have remission and exacerbation phases. RA and periodontitis show a degree of localization: in periodontitis not all teeth may be affected and RA may not affect all joints. Both diseases exhibit bone destruction and have been linked to vascular disease. Additionally, PMNs play a role in both diseases, producing a number of detrimental cytokines and enzymes, and hyperactive PMNs have been identified.

The evidence of a role for Th1 cells in the pathophysiology of RA is not strong and there is no evidence of a role for Th2 cells. IL-17 is strongly implicated in RA, but whether IL-17 is produced by Th17 cells or mast cells in the RA lesion is not clear. T regulatory cells (Treg) are also implicated in RA; a lower activity has been found in active lesions, while after successful treatment of RA, Treg activity is increased. In periodontal diseases, the development of gingivitis involves Th1 cells, while in periodontitis there is a shift towards Th2 cells (reviewed in Berglundh & Donati 2005). The number of Treg cells is increased in periodontitis compared with gingivitis tissues (Nakajima et al. 2005). Moreover, Th17 cells have been observed in periodontitis, but their role in the pathophysiology of this disease has yet to be determined (Yu et al. 2007, Gaffen & Hajishengallis 2008).

In summary, both RA and periodontitis present chronic inflammatory lesions adjacent to bone and in this context both diseases are characterized by connective tissue and bone destruction. While RA and periodontitis have different etiologies, individual steps in the pathophysiological pathways are common to both, presenting opportunities for the periodontal research community.

2. Is there any association between periodontitis and RA?

There is conflicting evidence that periodontitis and RA are associated. One study has shown that with increasing severity of RA, the odds of also having periodontitis increase, following adjustment for several potential confounding factors (De Pablo et al. 2008). In contrast, another recent study

showed no association (Arkema et al. 2010). Further epidemiological studies are required to resolve this discrepancy and must be supported by plausible biological mechanisms.

3. What are the most important targets to consider in the therapy of RA and is this relevant to therapy in periodontitis?

The most proven cytokine-targeted therapies for RA are anti-TNF-α and anti-IL-6, while anti-IL-1 has disappointing results. In periodontitis, therapies targeting TNF-α and IL-1β have demonstrated efficacy in animal models (Zhang et al. 2004, Di Paola et al. 2007), however no human studies have been reported. Interestingly, a limited number of small studies report on periodontal status in RA patients undergoing treatment for RA: TNF-α inhibition in these patients with RA was reported to either enhance, or have no effect on gingival inflammation, although reduced alveolar bone loss was noted. On the other hand, it has been suggested that nonsurgical treatment of periodontitis in patients with RA improved response to anti-TNF-α therapy.

Anti-B-cell ($CD20^+$) and anti-T-cell (CTLA4-Ig) therapies have been shown to be effective in treatment of RA, however they have not been investigated in relation to periodontitis.

Clinical studies to treat periodontitis with proven therapies in RA need to be considered.

Concluding Remarks from the Group

Many aspects regarding the pathophysiology of periodontitis are still unknown. To make significant progress for the benefit of science and public oral health, there is a need to create national and international research consortia. Only large collaborative efforts will allow the generation of sufficiently powered and well designed clinical and laboratory studies. Strong collaborations with non-dental scientists and the conviction that periodontal disease can serve as a very useful and accessible model for a bacterial driven inflammatory disease, will make it possible to elucidate the pathophysiology of periodontitis, with the translational effects on diagnosis, classification, prevention and the development of advanced medicine for oral health. The progress in the other disciplines of human medicine in recent years proves that this path is effective and worth the efforts.

In assessing the new evidence developed in the four reviews it is clear that the host response to the microbial biofilm in periodontal disease is highly complex and considerable individual variation pertains in all aspects of innate, inflammatory and immune responses to the periodontal microbial biofilm. Thus in developing and responding to questions within this group's consensus statement many questions are beyond the scope of the current literature and remain as useful foci of future research. Thus our general consensus statements as outlined above, are to some extent a plea for additional research.

References

Arkema, E. V., Karlson, E. W. & Costenbader, K. H. (2010) A prospective study of periodontal disease and risk of rheumatoid arthritis. *Journal of Rheumatology* **37**, 1800–1804.

Berglundh, T. & Donati, M. (2005) Aspects of adaptive host response in periodontitis. *Journal of Clinical Periodontology* **32** (Suppl. 6), 87–107.

Buduneli, N. & Kinane, D. F. (2011) Host derived diagnostic markers related to soft tissue destruction and bone degradation in periodontitis. *Journal of Clinical Periodontology* **38** (Suppl. 1), 85–105.

Culshaw, S., McInnes, I. B. & Liew, F. Y. (2011) What can the periodontal community learn from the pathophysiology of rheumatoid arthritis (RA). *Journal of Clinical Periodontology* **38** (Suppl. 1), 106–113.

De Pablo, P., Dietrich, T. & McAlindon, T. E. (2008) Association of periodontal disease and tooth loss with rheumatoid arthritis in the US population. *Journal of Rheumatology* **35**, 70–76.

Di Paola, R., Mazzon, E., Muia, C., Crisafulli, C., Terrana, D., Greco, S., Britti, D., Santori, D., Oteri, G., Cordasco, G. & Cuzzocrea, S. (2007) Effects of etanercept, a tumour necrosis factor-alpha antagonist, in an experimental model of periodontitis in rats. *British Journal of Pharmacology* **150**, 286–297.

Ford, P. J., Gamond, J. & Seymour, G. J. (2010) Immunological differences and similarities between chronic periodontitis and aggressive periodontitis. *Periodontology 2000* **53**, 111–123.

Gaffen, S. L. & Hajishengallis, G. (2008) A new inflammatory cytokine on the block: rethinking periodontal disease and the Th1/Th2 paradigm in the context of Th17 cells and IL-17. *Journal of Dental Research* **87**, 817–828.

Nakajima, T., Ueki-Maruyama, K., Oda, T., Ohsawa, Y., Ito, H., Seymour, G. J. & Yamazaki, K. (2005) Regulatory T-cells infiltrate periodontal disease tissues. *Journal of Dental Research* **84**, 639–643.

Preshaw, P. M. & Taylor, J. J. (2011) How has research into cytokine interactions and their role in driving immune responses impacted our understanding of periodontitis? *Journal of Clinical Periodontology* **38** (Suppl. 1), 60–84.

Nussbaum, G. & Shapira, L. (2011) How has neutrophil research improved our understanding of periodontal pathogenesis? *Journal of Clinical Periodontology* **38** (Suppl. 1), 49–59.

Wilson, C., Schulz, S. & Waldman, S. A. (2007) Biomarker development, commercialization, and regulation: individualization of medicine lost in translation. *Clinical Pharmacology & Therapeutics* **81**, 153–155.

Yu, J. J., Ruddy, M. J., Grace, C., Wong, G. C., Sfintescu, C., Baker, P. J., Smith, J. B., Evans, R. T. & Gaffen, S. L. (2007) An essential role for IL-17 in preventing pathogen-initiated bone destruction: recruitment of neutrophils to inflamed bone requires IL-17 receptor–dependent signals. *Blood* **109**, 3794–3802.

Zhang, X., Kohli, M., Zhou, Q., Graves, D. T. & Amar, S. (2004) Short- and long-term effects of IL-1 and TNF antagonists on periodontal wound healing. *Journal of Immunology* **173**, 3514–3523.

Address:
Denis F. Kinane
University of Pensylvania
School of Dental Medicine
240 South 40th Street
Philadelphia, PA 19104-6030
USA
E-mail: dfkinane@dental.upenn.edu