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How has research into cytokine interactions and their role in driving immune responses impacted our understanding of periodontitis?

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Abstract

Objective: To review current knowledge on cytokine interactions and the cytokine-mediated links between innate and adaptive immunity that are relevant to the pathophysiology of periodontitis.

Materials and Methods: A structured review of the literature was undertaken to identify relevant research publications using a Medline search from 1950 to September 2010. The focus of the search was on the functional role of cytokines, i.e. their actions and responses relevant to the pathogenesis of periodontal disease rather than more descriptive studies of their expression in tissues and body fluids. It was not possible to conduct a traditional systematic review with a focussed question due to the heterogeneity of published research.

Results: There is enormous heterogeneity in the periodontal literature in terms of experimental approaches. We have the deepest understanding of the role of the pro-inflammatory cytokines [e.g. interleukin (IL)-1β, tumour necrosis factor-α, IL-6] with accumulating data on T-cell regulatory cytokines (e.g. IL-12, IL-18), chemokines and cytokines which mediate bone cell development and function (e.g. receptor activator of NF-κB ligand, osteoprotegerin). It is clear that there are multiple, overlapping and complex functional links between cytokines with regulatory control exerted at a number of levels and involving numerous cell types (both immune cells and resident cells in the periodontium).

Conclusion: Cytokines appear to interact functionally in networks in the periodontium and integrate aspects of innate and adaptive immunity. However, our understanding is far from complete, particularly how molecular and cellular pathways relate to disease pathogenesis. We should adopt consistent experimental approaches to gain better insight into the totality of cytokine networks and how they drive immune responses in the periodontium.

Key words: acquired immunity; cytokines; innate immunity; lymphocytes; networks; periodontitis

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Our understanding of periodontal pathogenesis has evolved over the years, and will continue to evolve as research extends the knowledge base that underpins the science of periodontology. Within living memory of many periodontal researchers alive today, our understanding has transformed from periodontitis being considered an almost ubiquitous condition in which the role of plaque as the sole aetiological factor was unquestioned to modern awareness of the role of inflammation together with consideration of individual risk and susceptibility. Landmark publications have resulted in paradigm shifts in our way of thinking about periodontitis, such as the initiating role of plaque bacteria in

gingivitis (Loe et al. 1965), histological investigations of inflammation in the periodontium (Page & Schroeder 1976), the recognition of variations in disease susceptibility between individuals (Loe et al. 1986) and the importance of the host response (Page et al. 1997). We now know that the major determinant of disease susceptibility is the host immune-inflammatory response to the subgingival biofilm. It is paradoxical that these defensive processes result in the majority of the tissue damage leading to the clinical manifestations of disease. The unique anatomy of the periodontium adds complexity to our understanding of the role of the biofilm and the immune-inflammatory responses in the periodontal tissues.

The inflammatory response is characterized by infiltration of the tissues by neutrophils, macrophages and lymphocytes, and the generation of high concentrations locally of cytokines, eicosanoids and other destructive mediators such as the matrix metalloproteinases (MMPs). A large number of publications have reported on studies in which the local levels of various cytokines in different periodontal conditions were measured. As a general rule, many of these studies were underpowered (small number of subjects and limited assays) and focussed on one mediator at a time (in the classical reductionist approach). This has been understandable for reasons of technical complexity and cost when attempting to measure multiple cytokines simultaneously. It is becoming increasingly clear, however, that cytokines do not function in isolation, but rather in complex networks involving both pro- and anti-inflammatory effects. To focus exclusively on single mediators in research studies could limit our progress in improving understanding of this common, complex, inflammatory disease. In this review, we will focus on cytokine interactions and the cytokine-mediated links between innate and adaptive immunity that are relevant to the pathophysiology of periodontitis. It should be noted that given the heterogeneity of studies, it has not been possible to conduct a formal systematic review. This paper is in three parts: Part 1 is a general overview of cytokine functions, particularly in relation to innate and adaptive immunity. Part 2 is a summarization of current knowledge regarding cytokine biology. Part 3 is a more detailed consideration of cytokine interactions and their role in innate and adaptive immune responses as relevant to periodontology.

Part 1: Overview of Cytokine Function

Cytokines are soluble proteins that bind to specific receptors on target cells and initiate intra-cellular signalling cascades resulting in phenotypic changes in the cell via altered gene regulation. They are effective at low concentrations, are produced transiently in the tissues and primarily act in the tissues in which they are produced. Cytokines induce their own expression in an autocrine or paracrine fashion and have pleiotropic effects on a large number of cell types. They play a fundamental role in inflammation including periodontal disease (Hughes 1995). Interleukin-1 (IL-1) has been the most studied cytokine to date. It was first described as osteoclast-activating factor (OAF) (Horton et al. 1972). In 1985, OAF was purified and the amino-terminal sequence was determined to be identical to that of IL-1β, and it was concluded that IL-1β is the major protein with OAF activity produced by stimulated peripheral blood mononuclear cells (PBMCs) (Dewhirst et al. 1985). Around the same time, two distinct, but related complementary DNAs (cDNAs) that encoded proteins sharing IL-1 activity were isolated from a macrophage cDNA library, and the two cDNAs of IL-1 were termed IL-1α and IL-1β (March et al. 1985). Recombinant forms of IL-1α and IL-1β were subsequently confirmed to have diverse biological effects such as bone resorption, fever, induction of prostaglandin synthesis, and augmented T-cell responses to antigen.

Evidence for the existence of control pathways (i.e. the first evidence of cytokine networks, although they were not described as such at the time) came with the identification of what was initially called the IL-1 inhibitor (Arend & Dayer 1990), and after cloning was renamed as IL-1 receptor antagonist (IL-1Ra) (Carter et al. 1990, Eisenberg et al. 1990). It was recognized that this antagonist could be important for the in vivo regulation of IL-1 activity, as it binds to the IL-1 receptor but does not transduce a signal. In periodontal research, a large number of studies were subsequently conducted that investigated (mainly) IL-1β concentrations in gingival tissue and gingival crevicular fluid (GCF) in patients with various periodontal conditions. It was concluded that IL-1β had a major role in the pathogenesis of periodontal disease on account of it being consistently detected at increased levels in gingival biopsy samples and GCF in periodontitis patients, often with decreased concentrations after treatment (Howells 1995). Furthermore, IL-1β was confirmed as a potent inducer of bone resorption and of connective tissue degradation via the induction of MMPs (Birkedal-Hansen 1993). More recent research has further revealed the complexity of IL-1β secretion, with two signals required: the first being a Toll-like receptor (TLR)-induced transcription of pro-IL-1β, for example binding of lipopolysaccharide (LPS) to TLR-4, and the second being an additional signal (e.g. extracellular ATP), which results in assembly of an inflammasome, activation of caspase-1 and secretion of mature cytokine. The inflammasome is a multiprotein complex that mediates action of caspase-1, which is essential for activation of IL-1β (as well as IL-18 and IL-33) and is a critical component of immune functioning (Latz 2010). Research regarding inflammasomes in periodontal disease is at a very early stage, although there is emerging evidence that the NALP3 inflammasome plays a role in mediating inflammatory responses in the periodontium (Bostanci et al. 2009, Yilmaz et al. 2010).

The role of other cytokines, prostanoids and destructive enzymes in periodontal pathogenesis also has been investigated. Tumour necrosis factor-α (TNF-α) is a pro-inflammatory cytokine that induces bone resorption and upregulates prostaglandin E_2 (PGE_2) and MMP secretion. IL-1β and TNF-α are produced by many cell types in the periodontium; they induce upregulation of adhesion molecules on leucocytes and endothelial cells, they stimulate the production of chemokines (which recruit circulating leucocytes to sites of inflammation) and they induce expression of other inflammatory mediators that potentiate inflammatory responses, such as the prostaglandins and MMPs (Graves & Cochran 2003). In the mid-1990s, a sequence of pro-inflammatory events in the periodontium was described in which bacterial products such as LPS induce expression of IL-1β and TNF-α which in turn stimulate the production of cytokines, chemokines

and cyclooxygenase (COX) products which amplify the inflammation (Offenbacher 1996). Subsequently, MMPs are induced which break down connective tissue, and osteoclastic bone resorption commences, resulting in destruction of alveolar bone as the ''inflammatory front'' progresses deeper into the tissues. In terms of control of cytokines, it was recognized that under normal homeostasis, there is a degree of balance between pro- and anti-inflammatory activity, with specific T-cell cytokines, receptor antagonists such as IL-1Ra, inhibitory soluble forms of IL-1 and TNF receptors and the tissue inhibitors of metalloproteinases (TIMPs) all having some form of protective effect. It is noteworthy that in 1995, it was hypothesized that ''destructive periodontal disease may be due to dysregulation of these inhibitors, rather than an overproduction of IL-1 and TNF-α per se'' (Howells 1995) hinting at the presence of networks of cytokines functioning as part of inflammatory responses.

Cytokines and innate immunity

The actions of pro-inflammatory cytokines underpin the clinical signs and symptoms of immune responses (inflammation). Investigations of these molecules have dominated periodontal research for almost three decades and the most significant development has been the recognition of the fundamental role of innate immunity in initiating immune responses and regulating adaptive (antigen-specific) responses (Medzhitov 2010b). Periodontal research has enriched the general literature as periodontitis is driven by a diversity of bacterial pathogens, which harbour unusual variants of LPS which activate immunity using a variety of overlapping but subtly different pathways (Barksby et al. 2009, Hajishengallis 2009b). Further, individual bacteria (such as *Porphyromonas gingivalis*) can modify the biochemical structure of LPS in response to environmental conditions with concomitant effects on their interactions with the host innate immune responses (Darveau 2009).

LPS is one of a range of microbe-associated molecular patterns (MAMPs) which are recognized by host cells and which elicit cytokine responses. DNA, fimbriae and proteases are also capable of stimulating host immunity (Hajishengallis 2009a, Taylor 2010). In biological terms, the existence of a multitude of MAMPs within individual pathogenic species may compromise host defences and promote infection (Darveau 2009, Hajishengallis 2009a). Another important principle is that the innate immune response recognizes and responds to all colonizing microbes, both commensals and pathogens. There is strong evidence that commensal bacteria stimulate a low-level cytokine response in the periodontium necessary for priming host immunity and maintaining tissue integrity, and the immune response is amplified in response to changes in the microbial composition of plaque in which pathogenic bacteria dominate (Handfield et al. 2008, Darveau 2009, Taylor 2010).

MAMPs are detected by pattern recognition receptors (PRRs) such as the TLRs and their intra-cellular signalling pathways are highly conserved, which is testament to their critical importance to host integrity. It is now recognized that there are 10 functional TLR molecules in humans, which is consistent with the range of MAMPs expressed by infective microorganisms. The most studied pathway is recognition of LPS by a macromolecular complex involving CD14, MD-2 and TLR-4, the subsequent activation of intra-cellular signalling pathways leading to the translocation of the transcription factor NF-κB to the nucleus and consequent synthesis of pro-inflammatory cytokines including TNF-α and IL-1β. Most studies have focussed on cell surface receptors such as TLR-2 and TLR-4 but other TLRs are intra-cellular and occupy the cytoplasm either constitutively or as a result of internalization as part of the signalling process, e.g. the nucleic acid sensing TLR-7 and TLR-9 (Kawai & Akira 2010). Furthermore, other classes of PRRs, e.g. Nod-like receptors (NLRs), have been recognized and these receptors have important roles in sensing intra-cellular infections (Franchi et al. 2008). It is known that the NLR molecules NOD1/2 are expressed in oral epithelial cells (Uehara & Takada 2008) and NOD1/2 agonists synergize with *P. gingivalis* proteases to induce IL-6 and IL-8 secretion from monocytes (Uehara et al. 2008).

Tissue macrophages and dendritic cells (DCs) play a key role in the initial sensing of microbes. These cells are present in the periodontium and harbour the necessary array of PRRs. A number of studies have defined DC subsets in the periodontium and their potential role in periodontitis (Cutler & Jotwani 2006). There is heightened interest in monocytes as circulating cells functional in microbe recognition as these are precursors of macrophages, DCs and osteoclasts. Certain subsets of circulating monocytes may represent a ''hyperinflammatory'' phenotype and exhibit a distinct cytokine secretion profile (Auffray et al. 2009). However, there are only very limited data on monocyte subsets in periodontal disease (Nagasawa et al. 2004). The epithelial tissues of the mouth and periodontium also play an important role in host defences. Epithelial cells are essential elements of tissue integrity at mucosal surfaces and they respond to bacteria by upregulating cytokine secretion and expressing adhesion molecules (Swamy et al. 2010). Many periodontal bacteria invade and colonize epithelial cells endorsing the importance of intracellular PRRs and the signalling pathways they activate (Suchett-Kaye et al. 1998, Andrian et al. 2006). Fibroblasts are also a prominent cell type within the periodontium and are capable of mounting cytokine responses that perpetuate and amplify inflammation (Bartold & Narayanan 2006).

Activation of innate immunity is a prerequisite to the initiation of adaptive immunity, but may also lead to destructive (chronic) inflammation if the original insult persists. The importance of cytokines in the pathogenesis of periodontal disease is apparent at a number of levels. Not only do they act as initiators and regulators of innate and adaptive immunity but they also mediate the tissue damage, which leads to loss of function and clinical disease. The persistent activation of immune responses leads to inappropriate cytokine synthesis and secretion with concomitant effects on function and turnover of periodontal cells. Moreover, many of the non-immune cell types of the periodontium (e.g. keratinocytes and fibroblasts) synthesize cytokines in response to bacteria and other cytokines, and cytokines also influence turnover of extracellular matrix components and the fibres of the periodontal ligament (PDL) (Liu et al. 2010). Cytokines also have a central role in osteoclast activation (Bartold et al. 2010).

Inflammation is a response not restricted to infections (Medzhitov 2010a), and the concept of ''sterile inflammation'' has emerged, which can develop as a result of localized tissue

breakdown, cell ''stress'' or cell death (Iwasaki & Medzhitov 2010, Rock et al. 2010). For example, chromatin fragments released as a result of inefficient apoptosis may trigger TLR signalling (Marshak-Rothstein & Rifkin 2007). Also, breakdown products of extracellular matrix components, such as biglycan and hyaluronic acid, can activate macrophages via TLR-2 and TLR-4 to signal cytokine secretion (Jiang et al. 2005). The extent to which this occurs in the periodontium during periodontal disease has not yet been investigated. It is possible that inflammation and tissue damage in the periodontium result from the response not only to bacteria but also to cell contents and tissue breakdown products, and that a self-sustaining positive feedback circuit develops leading to loss of tissue function and exacerbated clinical disease.

Cytokines and adaptive immunity

Whereas innate immunity comprises macrophages, DCs, neutrophils, monocytes, epithelial cells and endothelial cells that identify and respond temporarily to MAMPs; the adaptive immune response relies on T- and B-cell recognition of specific antigen structures, resulting in immune responses which are highly specific and sustained (by the generation of immunological memory). Combinations of cytokines generated by macrophages and DCs create a milieu, which determines the differentiation of particular effector T-cell subsets as well as the class and subclass of immunoglobulin (Ig) antibodies synthesized. Cytokines do not achieve this in isolation, but act in concert with other signalling pathways and, in particular, cell-to-cell interactions via antigen presentation and co-stimulatory molecule function. Cytokines generated in these latter interactions are important to T-cell function and T-cell differentiation; naïve T-cells may differentiate into T helper 1 (Th1) cells, or into Th2 cells to provide ''help'' to B-cells, or into Th17 cells, which may amplify pro-inflammatory responses, or into regulatory T-cells (Treg cells), which dampen down immune responses. Th17 cells secrete IL-17 cytokines (which have a number of pro-inflammatory activities in common with IL-1β and TNF-α) and IL-22, and are important in immunity against extracellular bacteria and contribute to inflammation (Korn et al. 2009). Treg cells secrete transforming growth factor-β (TGF-β) and IL-10 and have a role in regulating other T-cell subsets and maintaining tolerance against self-antigens, thereby preventing autoimmunity (Josefowicz & Rudensky 2009). Foxp3 is an X-linked transcription factor belonging to the forkhead family that is essential for the development and function of Treg cells (Fontenot et al. 2003).

Functional T-cell subsets are broadly defined on the basis of the expression of cell surface molecules (CD4 or CD8) or particular T-cell antigen receptors. It has been proposed that alterations in the balance of effector $CD4^+$ T-cell subsets within the $CD4^+$ population may be a key event in disease progression (Gemmell et al. 2007). Cytokines have a central role in regulating the development of effector $CD4^+$ Th cell subsets and mediating their function. The original reports of the phenotypic dichotomy of $CD4^+$ T-cells described Th1 cells, which secrete interferon-γ (IFN-γ) and promote cell-mediated immunity by activating macrophages, natural killer (NK) cells and cytotoxic $CD8^+$ T-cells, whereas Th2 cells secrete IL-4, IL-5 and IL-13 and regulate humoral (antibody-mediated) immunity and mast cell activity (Mosmann & Coffman 1989). It was suggested that there is a dynamic interaction between T-cell subsets which may result in fluctuations in disease activity and that a Th1 response (providing protective cell-mediated immunity) underlies a ''stable'' periodontal lesion, and a Th2 response (leading to activation of B-cells) mediates a destructive lesion possibly through enhanced B-cell-derived IL-1β (Seymour & Gemmell 2001, Gemmell et al. 2007). However, there is no consistent evidence for the existence of distinct Th1 and Th2 cell populations in periodontal pathogenesis and it is increasingly recognized that the Th1/Th2 model alone is inadequate to explain the role of T-cells in this process (Gaffen & Hajishengallis 2008). This is likely because of variations in experimental studies, particularly the material that has been investigated, the design of the studies, the definitions of disease and the analytical methods that have been used (Houri-Haddad et al. 2007, Seymour et al. 2009). Furthermore, it has been shown that T-cells infiltrating the gingival tissues in patients with periodontitis can express mRNA for both Th1 and Th2 cytokines as well as regulatory cytokines simultaneously (Ito et al. 2005).

Cytokines play a major role in the regulation of T-cell subsets and it is clear that they act at a number of levels. For example, certain cytokines seem to be necessary but not sufficient by themselves for differentiation of specific T-cell subsets as exemplified by the requirement for IL-12 for induction of Th1 cells. Other cytokines reinforce the development of T-cell subsets already committed to a particular lineage and have an adjunct role in their differentiation, for example, IL-18 and IL-2 reinforce Th1 commitment (Murphy & Stockinger 2010). Further, T-cells are considered to exhibit ''functional plasticity'' and this phenomenon is influenced by the cytokine milieu (Bluestone et al. 2009). For example, under the influence of IL-12, Th17 cells can differentiate into Th1 cells (Korn et al. 2009), and follicular T-cells (T_{FH}), which reside in the B-cell follicles of lymph nodes, are dependent on IL-6 and IL-21 for their development, and can potentially secrete a cytokine profile corresponding to Th1, Th2 or Th17 cells (Vogelzang et al. 2008). The cellular context of developing T-cells is also important as cells other than antigen presenting cells (APCs) can act as a source of cytokines that influence T-cell differentiation. For example, NK cells are an important source of IFN-γ for Th1 differentiation (Martin-Fontecha et al. 2004), emphasizing the importance of innate cell instruction of adaptive immune responses. Caution should be used, however, as many of the recent studies on novel $CD4^+$ T-cell subsets have been limited to murine models and in vitro experiments, and the role of $CD4^+$ T-cell subsets in human immunology remains to be fully elucidated.

Cytokines also play a role in B-cell development and function. The key developmental stages of B-cells (in the follicle of secondary immune tissues such as lymph nodes) to antibody-producing plasma cells include activation and proliferation, selection of differentiation fate (to resident or peripheral plasma cells or into memory B-cells), qualitative aspects of antibody secretion (e.g. class switching and somatic hypermutation) and finally, plasma cell longevity (Goodnow et al. 2010). This process is influenced by a number of factors including antigen engagement, T-cell co-stimulation, signalling via

PRRs and the influence of the cytokine milieu. Chemokines and chemokine receptors are central to the distribution of lymphocytes including B-cells in secondary lymphoid tissues. IL-2, IL-4, IL-6 and IL-21 are key cytokines in regulating differentiation and reinforcing other signals delivered to B-cells (Shapiro-Shelef & Calame 2005). IL-5, IL-6 and TNF-α can all promote plasma cell survival and the presence of inflammation can therefore provide signals that maintain plasma cells in peripheral tissues (Cassese et al. 2003). B-cells and differentiated plasma cells characterize the established lesion in periodontal disease and high levels of antibodies appear in the GCF, but the significance of specific antibodies in the pathogenesis of periodontitis is, as yet, unclear (Barbour et al. 2009).

Part 2: Cytokine Biology

In order to better understand cytokine interactions in periodontal pathogenesis, current knowledge regarding cytokine biology is summarized in Table 1. This table provides brief details about the formation, cellular sources, receptors, signalling pathways, control mechanisms and function for selected cytokines believed to be important in periodontal pathogenesis. The number of identified cytokines continues to grow. For example, the IL-1 family now includes the ''traditional'' IL-1 cytokines (IL-1α, IL-1β, IL-Ra), IL-18, the so-called ''novel'' IL-1 cytokines (IL-1F5 to IL-1F10), and IL-33. These are all related to each other by amino acid sequence, receptor structure and signal transduction pathways, and they probably arose from duplications of a common ancestral gene (Arend et al. 2008, Sims & Smith 2010). The six ''novel'' members of the IL-1 cytokine family were identified on the basis of sequence homology, gene location, receptor binding and three-dimensional protein structure (Barksby et al. 2007). Table 1 reveals that cytokines are a diverse group of molecules with potent biological activity whose primary function is regulation of immune responses. It is likely that other molecules may be identified which have cytokine-like activity, as many genes encode proteins with no currently known function. For example, the gene for IL-32 was identified in 1992 (Dahl et al. 1992) but isolation of IL-32 protein (which has potent IL-1β and TNF-α-inducing properties) occurred much later (Kim et al. 2005).

Given the profound consequences of uncontrolled cytokine release, it is clear that regulation of these biologically active molecules is an essential part of inflammatory responses and immune functioning (Graves 2008). Cytokines function in networks of agonists, antagonists, receptors and receptor decoys, all of which regulate the activity of these powerful molecules. As an example, the agonist members of the IL-1 cytokine family are certainly extensively regulated (Sims & Smith 2010). IL-1β is inhibited by the receptor antagonist IL-1Ra and the decoy receptor IL-1R2. IL-18 is regulated by its binding protein, IL-18BP, and also is inhibited by IL-1F7. The agonist activities of IL-1F6, IL-1F8 and IL-1F9 are regulated by IL-1F5, an antagonist with similar structure to IL-1Ra that binds to IL-1RL2. IL-1F7 is anti-inflammatory, down-regulating cytokine release in LPS-stimulated cells. While the role of IL-1F10 is not clear, it has similar structure to IL-1Ra (and therefore also IL-1F5), so is likely to have antagonist activity. Given the complexity of cytokine interactions and regulation, periodontal pathogenesis is likely to be far more complex than previously thought.

Part 3: Cytokine Interactions and Immune Regulation in Periodontal Pathogenesis

The overall aim of this part of the paper is to review the evidence relating to cytokine interactions and their role in immune regulation in human periodontal pathogenesis. In order to identify relevant literature, a literature search was undertaken as described in Table 2. Given the nature of the subject area, and the inability to form a focussed question as would be typically done if performing a systematic review, it is clear that this literature search cannot be considered to be a systematic review. However, it is hoped that by following a structured and logical approach, the majority of the relevant literature has been identified. The overall aim of the search was to identify research publications that considered cytokine interactions and the cytokine-mediated links between innate and adaptive immunity that are relevant to the pathophysiology of periodontitis. Electronic database searches of Medline were performed from 1950 up to and including September 2010 using MeSH terms and keywords during the search (Table 2). The numbers of papers identified are indicated in Table 3, with 259 papers finally identified from the search strategy. Papers were excluded during the detailed scrutiny of titles and abstracts for many reasons, usually because they were not relevant to the purpose of this review (e.g. they focussed on something else such as implants, microbiology, endodontics, smoking), or were review papers, or were identified earlier in the sequential searching process. Furthermore, we did not wish to include every study that has investigated the levels of any particular cytokine in periodontal disease, as many of these papers have reported on studies of single mediators in various states of periodontal disease and health. These papers do not usefully inform on the importance of cytokine interactions and immune functioning as relevant to this review, and therefore they were not included. For information, and to demonstrate the heterogeneity of the studies, the 259 identified papers are listed in Table S1, and of these, 114 (44%) were considered relevant to this review and are cited in this paper. Three major concerns were identified when undertaking the literature search:

1. A disproportionately large number of review papers were identified, for example 183 of 573 papers (32%) that were identified in the broad (cytokines AND periodontal diseases) search were review papers.
2. No common research strategy appears to be evident, i.e. there appears to be no broad focus on resolving a single major issue. There are few common themes of research, and the various research groups are performing their own highly specific research in the context of their own developed research models. This results in significant heterogeneity of the published studies because methodologies, experimental systems and analytical techniques all vary widely and drawing simple, clear conclusions is therefore extremely difficult.
3. There is a relatively small amount of published research using human cells and tissues; much of the research in

Table 1. Summary of cytokine biology

Cytokine	Formation/cellular sources	Receptors and signalling	Control mechanisms	Function	References
IL-1α, IL-1β, IL-1Ra	Pro-IL-1α (biologically active) is synthesized in cytoplasm and is cleaved by calpain to generate IL-1α, which remains within the cell. Pro-IL-1β (biologically inactive) is synthesized in cytoplasm and is cleaved by caspase-1 to generate IL-1β. sIL-1Ra (secretory IL-1Ra), the major isoform of IL-1Ra, is synthesized in the Golgi. All are synthesized by multiple cells including monocytes, macrophages, neutrophils, keratinocytes, epithelial cells and fibroblasts.	The IL-1 receptors are the type 1 and two IL-1 receptors (IL-1R1 and IL-1R2) and IL-1R accessory protein (IL-1RAcP). When IL-1 binds to IL-1R1, IL-1RAcP forms a complex with the bound IL-1/IL-1R1. Adaptor molecules are recruited by this complex (MyD88, IRAK, TRAF6), which activate transcription factors (NF-κB, AP-1, JNK and p38 MAPK).	Binding of ligand (IL-1α, IL-1β) to IL-1R1 recruits signal transduction proteins and activates cells but binding to IL-1R2 does not (i.e. IL-1R2 competes with IL-1R1 and functions as a decoy receptor for IL-1). IL-1Ra binds to IL-1R1 and IL-1R2 but cannot interact with IL-1RAcP and therefore cannot activate cells (i.e. IL-1Ra inhibits IL-1).	IL-1α: pro-inflammatory, located in the membrane rather than secreted. Acts as an intra-cellular transcriptional regulator. IL-1β: pivotal and wide-ranging function in innate immunity and inflammation. Regulates adaptive immunity (T-cells and myeloid cells) and stimulates connective tissue turnover. IL-1Ra: antagonizes IL-1α and IL-1β action.	Akira et al. (2006) Arend et al. (1998) Arend et al. (2008) Barksby et al. (2007) Colotta et al. (1993) Dinarello (1997) Dinarello (1998a) Dinarello (1998b) Dinarello (2008) Dinarello (2009) Sims (2002)
IL-18	Pro-IL-18 (inactive precursor) is cleaved by caspase 1 to generate IL-18. Produced by various cells types including monocytes, macrophages, dendritic cells, epithelial cells, keratinocytes The binding chain (IL-18Rα) is expressed on naïve T-cells, Th1 lymphocytes, NK cells, macrophages, B-cells, neutrophils and epithelial cells. IL-18 binding protein (IL-18BP) is produced by endothelial cells, monocytes and macrophages.	The IL-18 receptor (IL-18R) complex is homologous to the IL-1R1 complex and requires IL-18R accessory protein (IL-18RAcP) for signalling. The binding chain of the complex is a heterodimeric protein consisting of IL-18Rα (the non-signalling moiety that binds IL-18) and IL-18Rβ (the signalling moiety that forms a complex with IL-18/IL-18Rα). The cytoplasmic tail of IL-18Rβ recruits the same adaptor molecules as IL-1 (MyD88, IRAK, TRAF6).	IL-18BP is a soluble protein that functions as a decoy receptor for IL-18 binding. Production of IL-18BP is increased by IFN-γ, thereby inhibiting IL-18 in a feedback loop as IL-18 drives Th1 responses.	Pro-inflammatory role alongside IL-1β, and amplifies immune responses by inducing other cytokines (IL-1β, TNF-α, IL-8, GM-CSF). IL-18 can drive both Th1 and Th2 responses. In the presence of IL-12, it drives IFN-γ-producing Th1 cells. If IL-12 is absent, it can drive IL-4-producing Th2 responses. Stimulates neutrophil migration/activation and osteoclastic activity, and is important in removal of intra-cellular pathogens and viruses (via induction of IFN-γ and cytotoxic T-cells).	Arend et al. (2008) Dinarello (2000) Dinarello (2009) Gracie et al. (2003) Komai-Koma et al. (2003) Liu et al. (2010) Paulukat et al. (2001) Puren et al. (1998) Sims (2002) Weaver et al. (2006)
IL-1F5, IL-1F6, IL-1F7, IL-1F8, IL-1F9, IL-1F10	These IL-1 cytokines are expressed particularly in skin and can be induced in monocytes. Their receptor (IL-1RL2) is highly expressed on epithelial cells in the skin and gastrointestinal tract. IL-1F7 precursor is cleaved by caspase-1 and the active form translocates to the nucleus where it may modulate transcriptional	All six interact with the same receptor, IL-1RL2 (originally referred to as IL-1Rrp2). When IL-1F6, IL-1F8, IL-1F9 bind to IL-1RL2, IL-1RAcP is recruited to form a heterodimer, leading to NF-κB activation. IL-1F7 is structurally similar to IL-18 and also binds to IL-18BP and IL-18R. There are five isoforms of IL-1F7.	IL-1F5 is antagonist and binds to IL-1RL2 thereby blocking binding of the agonists (IL-1F6, IL-1F8, IL-1F9). IL-1F5 is structurally similar to IL-1Ra. IL-1F7 is an intra-cellular regulator and inhibits production of IL-1β, TNF-α and IL-18 (possibly by binding	IL-1F6, IL-1F8, IL-1F9 are all pro-inflammatory and activate NF-κB and MAPK. They have restricted expression: IL-1F6 (skin), IL-1F8 (skin, synovial tissues), IL-1F9 (skin, placenta, oesophagus). IL-1F5 directly antagonizes IL-6 action, and has anti-inflammatory effects via IL-4 induction. IL-1F7 is an intra-cellular anti-inflammatory	Arend et al. (2008) Barksby et al. (2007) Blumberg et al. (2007) Bufler et al. (2002) Costelloe et al. (2008) Dinarello (2009) Sharma et al. (2008) Sims et al. (2001) Sims & Smith (2010) Towne et al. (2004)

Table 1. (Contd.)

Cytokine	Formation/cellular sources	Receptors and signalling	Control mechanisms	Function	References
	activity, reducing the production of LPS-stimulated cytokines.	IL-1F10 shares one-third of its amino acid sequence with IL-1Ra, and recombinant IL-1F10 binds to IL-1R1.	IL-18BP thereby preventing its interaction with IL-18Rα).	regulator, and decreases cytokine production, particularly IL-18. IL-1F10 is likely to function as a receptor antagonist (like IL-1Ra), but its precise role is unclear.	
IL-33	IL-33 is produced as a biologically active pro-peptide that is possibly cleaved by caspase 1. It is structurally similar to IL-18 and is considered to be an IL-1 cytokine. IL-33 has restricted expression (endothelial cells, smooth muscle cells, fibroblasts).	ST2 is the receptor for IL-33. Binding of IL-33 to ST2 induces signalling through the NF-κB and MAPK pathways, and IL-1RAcP has been reported to be required for signalling. The ST2 receptor is expressed on Th2 cells, mast cells, epithelial cells and some mesenchymal cells.	A soluble form of ST2 (sST2) acts as an antagonistic decoy receptor for IL-33, and is produced by fibroblasts, macrophages and monocytes that are stimulated by IL-1β, TNF-α or LPS, providing control over IL-33 activity.	IL-33 is pro-inflammatory. It drives the secretion of Th2 cytokines from Th2 cells, including IL-4, IL-5 and IL-13. It is chemoattractant for Th2 cells, and induces production of inflammatory mediators such as IL-6, IL-1β, TNF-α. IL-33 may mainly have an intra-cellular function in monocytes, acting as an ''alarmin'' to signal cellular damage when released from damaged or necrotic cells.	Ali et al. (2007) Arend et al. (2008) Barksby et al. (2007) Blumberg et al. (2007) Carriere et al. (2007) Dinarello (2009) Hayakawa et al. (2007) Komai-Koma et al. (2007) Nile et al. (2010) Schmitz et al. (2005) Sims & Smith (2010)
TNF-α	TNF-α is produced as pro-TNF-α, which is expressed on the plasma membrane and then cleaved extracellularly by MMPs, resulting in the soluble form, TNF-α. Both membrane-bound and soluble forms possess biological activity. TNF-α-converting enzyme (TACE) mediates release of TNF-α from cell surfaces and also processes TNF-α receptors, resulting in their release as soluble forms that bind TNF-α. TNF-α is mainly produced by macrophages and T lymphocytes, but also by neutrophils, B-cells, fibroblasts, osteoclasts and endothelial cells.	The two TNF receptors are TNFR1 and TNFR2, which recruit intra-cellular cytosolic proteins to activate signal transduction pathways. Pro-inflammatory effects of TNF-α are mainly mediated through TNFR1 (by recruitment of TRADD and subsequent activation of MAPKs as well as NF-κB). Signalling via TNFR2 appears to be linked to angiogenesis and tissue repair.	TACE can exert both pro- and anti-inflammatory activity depending on whether it releases ligand (i.e. TNF-α) or soluble TNF-α receptors. TNFR1 and TNFR2 utilize different signalling mechanisms, permitting distinct biological responses depending on their relative expression. TNF-α, IL-1β and IL-10 increase expression of TNFR2 and tend to decrease expression of TNFR1.	TNF-α is a key regulator of immune and inflammatory responses. Pro-inflammatory effects of TNF-α mainly result from its effects on endothelial cells and their interaction with leucocytes. TNF-α causes increased expression of ICAM-1, VCAM-1 and E-selectin which together with release of chemokines (e.g. IL-8) results in recruitment of leucocytes. TNF-α also induces expression of COX-2 in endothelial cells, leading to vasodilatation and increased vascular permeability.	Bradley (2008) Carswell et al. (1975) De Trez & Ware (2008) Kim et al. (2005) Locksley et al. (2001) Mark et al. (2001) Ware (2003) Ware (2008)
IL-6	IL-6 is produced by a range of immune and resident cells including T-cells, B-cells, macrophages, osteoblasts, DCs, keratinocytes, endothelial cells, fibroblasts, adipocytes. It is produced from a single gene encoding a product of 212 amino acids, which is cleaved	IL-6 family cytokines (IL-6, IL-11, LIF – leukaemia inhibitory factor, oncostatin M) share common signalling pathways via the signal transducers glycoprotein (gp) 130. IL-6 binds the IL-6 receptor (IL-6R), forming the IL-6/IL-6R complex that binds to membrane-bound gp130. This activates intra-	The SOCS family of proteins (suppressor of cytokine signalling) regulate gp130 signalling by inhibiting the JAK/STAT signal transduction pathways. Production of SOCS proteins occurs following cytokine stimulation, is induced by STAT-mediated gene	gp130 is expressed on many cell types, which explains the pleiotropic properties of IL-6. IL-6 has pro-inflammatory properties, and plays a key role in acute inflammation, and promotes bone resorption. It amplifies inflammation, stimulates fever, angiogenesis and acute phase	Alexander (2002) Bettelli et al. (2006) Blanchard et al. (2009) Eder et al. (2009) Fonseca et al. (2009) Heinrich et al. (2003) Kishimoto (2005) Nakashima & Taga (1998) Tamura et al. (1993)

	at the N-terminus to produce the active 184-amino acid peptide.	cellular pathways that include JAKs, STAT1, STAT3, NF-κB.	transcription, and inhibits cytokine signalling in a negative feedback loop.	protein release. It also stimulates T-cell differentiation and is important in the balance between T-cell subsets.	Van Snick (1990) Wong et al. (2003)
IL-12	IL-12 cytokines (IL-12, IL-23, IL-27, IL-35) are structurally related, and are secreted by monocytes, macrophages and DCs in response to infection. They are heterodimeric proteins, which share common subunits. IL-12 is composed of IL-12p35 and IL-12p40 subunits to form the biologically active IL-12p70. IL-23 is composed of IL-12p40 and IL-12p19 subunits. IL-27 is composed of EBI3 and p28 subunits. IL-35 is composed of IL-12p35 and EBI3.	The IL-12 receptor complex is formed by two chains, IL-12Rβ1 and IL-12Rβ2 that are homologous to gp130. IL-12Rβ1 is associated with Janus kinase (JAK) Tyk2 and binds IL-12p40; IL-12Rβ2 is associated with Jak2 and binds either the heterodimer or the p35 chain. IL-12, IL-23 and IL-27 all activate similar members of the JAK/STAT signalling pathways but have specific roles in regulating immune responses.	IL-12 is synergistically induced when more than one PRR is engaged. Cytokines such as IFN-γ and IL-4 also enhance IL-12 production. Anti-inflammatory cytokines are important in regulating IL-12 and IL-23 production. IL-10 is a potent inhibitor of IL-12 by blocking transcription of both its genes by induction of repressor proteins and preventing the transcription of NF-κB-induced genes.	IL-12 cytokines play a central role in activating T-cell responses. IL-12 secretion by myeloid cells results in the differentiation of $CD4^+$ T-cells into Th1 cells characterized by IFN-γ production. IL-23 has a role in maintenance of IL-17 secreting Th17 cells. IL-27 has an early role in the induction of Th1 differentiation in naïve $CD4^+$ cells. IL-35 is an anti-inflammatory cytokine produced by Treg cells which suppress Th17 cell activity.	Beadling & Slifka (2006) Collison et al. (2007) Gee et al. (2009) Kastelein et al. (2007) Korn et al. (2009) Lyakh et al. (2008) Trinchieri (2003) Yoshida & Miyazaki (2008)
IL-17	The IL-17 family includes IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (IL-25) and IL-17F. IL-17A is the most studied and is usually referred to as IL-17. IL-17 is produced by T-cells, macrophages, DCs, mast cells and NK cells. IL-17-producing $CD4^+$ T-cells (Th17 cells) arise as a distinct population from Th1 and Th2 cells. Th17 cells also produce TNF-α, IL-22 and IL-21.	The receptors are IL-17RA/IL-17R, IL-17RB/IL-15R, IL-17RC, IL-17RD/SEF and IL-17RE. IL-17R is expressed ubiquitously, meaning most cells can respond to IL-17. Signalling is not fully characterized, but binding of IL-17 to IL-17RA and IL-17RC recruits the adaptor protein TRAF6, leading to activation of NF-κB.	Control mechanisms are not fully elucidated. IL-17 synergizes with other cytokines, including IL-1β, TNF-α, oncostatin M and IFN-γ resulting in profound biological effects in vivo.	IL-17 has pro-inflammatory effects, causing bone resorption and inflammation in rheumatoid arthritis by inducing IL-1β, TNF-α and IL-6. IL-17 also stimulates chemokine release (IL-8) and induces expression of various MMPs. It also activates COX-2 and induces expression of RANKL in osteoblasts, leading to bone resorption.	Dorhoi & Kaufmann (2009) Gaffen (2009) Korn et al. (2009) Onishi & Gaffen (2010) Park et al. (2005) Steinman (2007) Weaver (2009) Yao et al. (1997)
Chemokines	Chemokines are divided into four sub-families based on molecular structure (CXC, CX3C, CC, C). The nomenclature has been revised according to the receptor nomenclature (CCL1, CXCL1, etc). Expression is stimulated by LPS and cytokines (e.g. IL-1β, TNF-α). Chemokines are produced by various resident and infiltrating cells (fibroblasts, osteoblasts, mast cells, epithelial cells, endothelial cells, neutrophils, lymphocytes and monocytes).	The receptors CCR1 and CCR5 (on monocytes/macrophages) bind MIP-1α/CCL3 and RANTES/CCL5. CCR2 binds MCP-1/CCL2. Neutrophils express CXCR1 and CXCR2 which bind IL-8/CXCL8, GCP2/CXCL6 and GROα/CXCL1. Chemokine receptor binding activates intra-cellular signalling pathways involving small GTPases (RAP1, RhoA, Rac) and kinases (PI_3K), activation of MAPKs, and NF-κB.	Chemokines are regulated at transcriptional, post-transcriptional, translational and post-translational levels. Pro-inflammatory chemokines are induced by cytokines such as IL-1β, TNF-α and IFN-γ. Others (e.g. those that play a role in normal leucocyte migration) are constitutively expressed.	Chemokines play a key role in inflammation by orchestrating the tissue distribution of leucocyte subsets in tissues and regulating cell migration and proliferation. Activation of signalling pathways by chemokine receptor binding results in reorganization of the cell cytoskeleton, resulting in pseudopodia which permit the cell to move up the chemotactic gradient.	Bonecchi et al. (2009) Kakinuma & Hwang (2006) Mantovani et al. (2006) Murphy (1996) Sharma (2010) Silva et al. (2007) Viola & Luster (2008) Yoshimura et al. (1987) Zlotnik et al. (2006)
RANKL/OPG	RANK is a cell surface receptor expressed by osteoclast progenitors and mature osteoclasts. RANK ligand (RANKL) binds to RANK	OPG is a decoy receptor for RANKL, and inhibits bone resorption by binding RANKL, thereby preventing it from binding	Osteoblasts control osteoclast behaviour via M-CSF and RANKL. M-CSF is secreted and binds to c-Fms on the surface of	Binding of RANKL to RANK results in osteoclast differentiation and activation, and thus bone resorption. IL-1β and TNF-α induce	Bartold et al. (2010) Bostanci et al. (2007) Boyle et al. (2003) Cochran (2008)

Table 1. (Contd.)

Cytokine	Formation/cellular sources	Receptors and signalling	Control mechanisms	Function	References
	and is expressed by bone marrow stromal cells, osteoblasts and fibroblasts, as well as other cell types such as fibroblasts, and T and B lymphocytes.	to RANK. Binding of RANKL to RANK on the surface of M-CSF-stimulated osteoclast precursors results in activation of signalling pathways mediated by TRAF-6, NF-κB and AP-1.	osteoclast precursors, resulting in their proliferation. RANKL triggers their differentiation into mature osteoclasts.	expression of both RANKL and OPG. Increased RANKL:OPG ratios result from IL-1β and TNF-α activation, and lead to increased bone resorption as more RANKL is available (relative to OPG) to bind RANK on osteoclast precursors.	Hughes et al. (2006) Katagiri & Takahashi (2002) Khosla (2001) Koide et al. (2010) Tyrovola et al. (2008) Vega et al. (2007)
IL-10	The main source of IL-10 is Treg cells but it is also produced by other T-cell subsets (Th1, Th2, Th17, $CD8^+$ T-cells), monocytes, macrophages, DCs and B-cells. Other members of the IL-10 superfamily include IL-19, IL-20, IL-22, IL-24, IL-26, IL-28 and IL-29.	IL-10 forms a homodimer and signals through the IL-10R1 and IL-10R2 receptor complex, with subsequent activation of signal transduction pathways including Jak 1, tyrosine kinase 2, STAT1 and STAT3.	Knowledge of IL-10 regulation is limited, and regulatory mechanisms differ between different cell types. In macrophages and DCs, ERK- and p38-dependent IL-10 production is inhibited by IFN-γ. Inhibition of p38 signalling in DCs and monocytes inhibits IL-10 expression. Transcriptional factors also regulate IL-10 (increasing or decreasing expression), indicating a genetic component to IL-10 control.	IL-10 has an anti-inflammatory role, and inhibits the activity of Th1 cells (inhibits IFN-γ, IL-2), Th2 cells (inhibits IL-4, IL-5), NK cells (inhibits IFN-γ, TNF-α) and macrophages (inhibits IL-1β, IL-6, IL-8, IL-12, TNF-α). IL-10 also inhibits expression of co-stimulatory molecules CD80 and CD86 by DCs and other APCs, preventing them from providing signals for Th cell activation. IL-10 has recently been shown to also possess pro-inflammatory effects through activating B-cell proliferation and Ig secretion, suggesting dual roles for IL-10.	Commins et al. (2008) Couper et al. (2008) Hedrich & Bream (2010) Larche (2007) Maynard & Weaver (2008) Saraiva & O'Garra (2010) Scumpia & Moldawer (2005) Wynn (2008)
TGF-β	TGF-β consists of a family of pleiotropic cytokines. There are three isotypes of TGF-β; TGF-β1, TGF-β2 and TGF-β3, all with similar biological activity. A variety of cell types produce TGF-β. TGF-β and Treg cells play a critical role in maintaining self-tolerance and immune homeostasis.	TGF-β binds the type II TGF-β receptor (TβRII), and the ligand-bound TβRII activates the type 1 TGF-β receptor (TβRI). Activation of signalling occurs through serine–threonine kinase activity, and downstream signalling is via SMAD-dependent and SMAD-independent pathways.	SMAD-dependent signalling can be repressed by complex association with other transcription factors and inhibitors. The balance of SMAD-dependent and SMAD-independent signalling, and the presence of signalling repressors determines the response to TGF-β.	TGF-β regulates multiple aspects of cellular functions, and is critical in suppressing immune responses. It regulates T-cell subsets and plays a role in repair, regeneration, angiogenesis, apoptosis and inhibition of cell growth. TGF-β can induce the production of IL-10 in Treg cells. It has inhibitory effects on Th1 cells, Th2 cells, NK cells, B-cells, PMNs, macrophages and prevents DC maturation.	Bierie & Moses (2010) Gordon & Blobe (2008) Larche (2007) Prud'homme (2007) Saraiva & O'Garra (2010) Wan & Flavell (2007a) Wan & Flavell (2007b) Wynn (2008)

IL, interleukin; TNF, tumour necrosis factor; Th, T helper; TGF, transforming growth factor; PMN, polymorphonuclear; NK, natural killer; DC, dendritic cell; APC, antigen presenting cell; GM-CSF, granulocyte macrophage colony-stimulating factor; ICAM, inter-cellular adhesion molecule; VCAM, vascular cell adhesion molecule; TRAF, TNF receptor-associated factor; OPG, osteoprotegerin; RANKL, receptor activator of NF-κB ligand.

Table 2. Search strategy

Search level	Search combinations	Search terms	*N* of studies	Comments
1		Periodontitis OR chronic periodontitis OR aggressive periodontitis OR periodont$ OR gingivitis OR periodontal disease(s) OR gingival disease(s) OR gingiva OR gingival tissue OR gingiv$ OR periodontal ligament OR PDL or alveolar bone OR alveolar process OR alveolar bone loss	69,176	This search identified papers in the literature that are relevant to periodontal diseases
2		Mediator of interest, as indicated in Table 3: each mediator was searched for in both the full and abbreviated name, and as a keyword and a title word	323,150	This search identified papers in the literature that focussed on the mediators of interest
3	(1)+(2)	The periodontal search terms (1) were combined with each mediator of interest (2) using an [AND] operation	2994	This identified papers in the periodontal literature that reported on the mediators of interest. Even after excluding reviews, many reviews were still present in the final reference list and had to be excluded during the review of titles and abstracts. Similarly, even after limiting studies to human studies (lines 8 and 12), many animal studies continued to be identified by the search engines
4	Limit (3) to English	The literature identified in (3) was limited to English language articles	2885	
5	Limit (4) to exclude reviews	Review articles excluded from (4)	2526	
6		Innate immunity OR monocytes OR macrophages OR monocytic cells OR dendritic cells OR epithelial cells OR keratinocytes OR fibroblasts OR endothelial cells OR neutrophils OR granulocytes OR mast cells	583,404	This search identified papers in the literature that are relevant to innate immunity
7	(2)+(5)+(6)	The identified periodontal literature (5) was combined with innate immunity literature (6), for each mediator of interest (2) using an [AND] operation	1144	This search identified periodontal literature that focussed on aspects of innate immunity according to the mediators of interest. Of the 995 papers, 782 papers were excluded as follows (including the primary reason for exclusion): 7 (focussed on implants); 44 (focussed on microbiology); 20 (focussed on smoking); 13 (were genetic association studies); 16 (were endodontic studies); 33 (focussed on DIGO); 43 (were review papers); 191 (were already prior identified in the sequential search strategy); 415 (were otherwise not relevant to this review)
8	Limit (7) to human studies	The periodontal/innate immunity literature limited to human studies only	995	
9	Abstract scrutiny	All papers identified in (8) reviewed for relevance to this review paper	**213**	
10		Adaptive immunity OR acquired immunity OR T-lymphocytes OR B-lymphocytes OR T-cells OR B-cells OR T-lymphocytes, regulatory OR T-lymphocyte subsets OR Th1 cells OR Th2 cells OR CD4:CD8 ratio OR CD4-positive T-lymphocytes OR CD8-positive T-lymphocytes	402,263	This search identified papers in the literature that are relevant to adaptive immunity
11	(2)+(5)+(10)	The identified periodontal literature (5) was combined with adaptive immunity literature (10), for each mediator of interest (2) using an [AND] operation	415	This search identified periodontal literature that focussed on aspects of adaptive immunity according to the mediators of interest. Of the 335 papers, 307 papers were excluded as follows (including the primary reason for exclusion): 1 (focussed on implants); 5 (focussed on microbiology); 15 (focussed on smoking); 3 (were genetic association studies); 4 (were endodontic studies); 2 (focussed on DIGO); 47 (were review papers); 144 (were already prior identified in the sequential search strategy); 86 (were otherwise not relevant to this review)
12	Limit (11) to human studies	The periodontal/adaptive immunity literature limited to human studies only	335	
13	Abstract scrutiny	All papers identified in (12) reviewed for relevance to this review paper	**28**	
14	Final broad survey	Cytokines	77,324	This final broad survey was performed to identify any papers that were missed in the previous searches. Of the total of 573 papers identified in the [AND] operation, only 18 additional papers were identified that were relevant to this review. Of the remaining 555, 73 had already been identified in the searches above, 183 were review papers, and 299 were not relevant to this review for various reasons
15	(1)+(14)	All periodontal literature (1) was combined with cytokines (14) using an [AND] operation	573	
16	Abstract scrutiny	All papers identified in (15) reviewed for relevance to this review paper	**18**	
Totals		The total number of papers identified in this search process is: 213 (identified in 9 above)+ 28 (identified in 13 above)+ 18 (identified in 16 above)	**259**	

Numbers of studies in the search strategy can be cross-referenced with the information in Table 3.

Table 3. Results of search strategy, numbers of papers identified

Mediator of interest	Total *N* of studies identified (Line 2)	*N* after AND with perio search terms (Line 3)	*N* after limit to English language (Line 4)	*N* after reviews excluded (Line 5)	*N* after perio AND innate search (Line 7)	*N* after limit to human studies (Line 8)	*N* after abstract scrutiny (Line 9)	*N* after perio AND adaptive search (Line 11)	*N* after limit to human studies (Line 12)	*N* after abstract scrutiny (Line 13)
IL-1α	5797	142	132	97	47	41	17	6	5	2
IL-1β	17,968	515	495	415	193	175	54	32	29	5
IL-1Ra	4671	53	50	43	11	10	3	0	0	0
IL-18	4453	34	34	28	12	11	3	6	5	2
IL-1F5 to -F10	21	3	3	1	1	1	0	0	0	0
IL-33	171	1	1	0	0	0	0	0	0	0
TNF-α	100,418	749	720	603	310	261	52	66	50	3
IL-6	58,678	613	590	491	239	221	41	62	54	7
IL-17	3579	35	35	131	12	10	3	21	17	4
IL-12	9783	39	39	38	19	10	6	21	16	1
Chemokines	36,163	207	203	177	116	103	15	37	34	1
RANKL/OPG	5080	316	302	255	58	43	8	43	33	1
IFN-γ	76,368	287	281	247	126	109	11	121	92	2
Totals	323,150	2994	2885	2526	1144	995	213	415	335	28

The line numbers in parentheses (Line 2, Line 3, etc.) refer to the search levels listed in Table 2.
IFN, interferon; IL, interleukin; OPG, osteoprotegerin; RANKL, receptor activator of NF-κB ligand; TNF, tumour necrosis factor.

this area has used various animal models of periodontal disease.

Signals that lead to cytokine secretion in periodontal disease

Developments in microbiology, molecular biology and microbial genomics have greatly enhanced our understanding of the structure and diversity of the microbial elements of dental plaque. A major challenge to improving our understanding of the signals that lead to cytokine secretion is that most studies have investigated a limited number of bacterial species and/or have focussed on single organisms; information on host signalling and cytokine secretion elicited by microorganisms in the context of biofilms is lacking. The recent application of microarray technology has enabled more holistic studies of host cell responses to bacteria that will improve our understanding (Mans et al. 2010). Significantly, bacteria that colonize the biofilm at different stages and which have different pathogenic potential influence similar host response pathways (Handfield et al. 2008). The biological consequences of this have yet to be fully described but there is evidence that individual species have characteristic effects on signalling pathways associated with a range of pro-inflammatory cytokines (IL-1α, IL-1β, IL-6 and IL-8) and T-cell stimulating cytokines (IL-12, IL-23). It is not clear how this information integrates into a model of periodontal pathogenesis but clearly there is potential to link bacterial diversity to intra-cellular signalling pathways and cytokine secretion profiles. Few comparative studies have been performed on cytokine profiles regulated by different LPS structures (as opposed to the many studies of individual mediators). However, two such studies, both comparing *P. gingivalis* and *Escherichia coli* LPS have identified important differences in chemokine gene expression patterns stimulated by different forms of LPS (Chen et al. 2007, Barksby et al. 2009).

In vitro experiments indicate that host cells respond to bacteria by activating intra-cellular signalling pathways leading to cytokine secretion (Handfield et al. 2008). Whole periodontal bacteria have been shown to stimulate the secretion of a range of pro-inflammatory cytokines such as IL-1α, IL-1β, IL-6, IL-8 and IL-12 (Sandros et al. 2000, Kusumoto et al. 2004). There is also the suggestion that some bacteria such as

P. gingivalis may inhibit IL-8 secretion (and therefore compromise host innate immunity) although it is not yet clear if this mechanism is important in vivo (Darveau et al. 1998, Huang et al. 2001). Little is known about interactions between cytokines and periodontal pathogens. Research has found that *P. gingivalis* can hydrolyse IL-1β, IL-6 and IL-1Ra, and thus can alter the local cytokine network not only by stimulating the release of cytokines from host cells, but also by removing them from the local environment (Fletcher et al. 1997).

The majority of in vitro studies of interactions between plaque bacteria and host cells have been performed using *P. gingivalis*. Clearly, this is an important pathogen and has provided useful paradigms for investigation of the properties of other plaque bacteria. *P. gingivalis* LPS is structurally distinct from *E. coli* LPS (Ogawa 1993), and it induces IL-1β, TNF-α and IL-6 production via a different signalling mechanism from that of *E. coli* LPS (Diya et al. 2008). Furthermore, *P. gingivalis* LPS is heterogeneous; some *P. gingivalis* LPS molecules bind to TLR-2 and some to TLR-4 and some are antagonists (Hajishengallis et al. 2002, Darveau et al. 2004). It is noteworthy that the structure of the lipid A moiety of *P. gingivalis* LPS is altered in response to nutrient availability, with consequent reduction in TLR-4 signalling, which may be a feature that has evolved to allow the bacterium to evade immune responses (Al-Qutub et al. 2006, Coats et al. 2009).

P. gingivalis has a number of other structural elements which can be considered as MAMPs on the basis of their ability to stimulate host immunity. It is well established that fimbriae (particularly the major form, FimA) are central to cell adhesion and activation of host responses by *P. gingivalis*. Fimbriae activate TLR signalling pathways resulting in production of IL-1β, IL-6, TNF-α and IL-8 in monocytic cells (Hajishengallis et al. 2004). Endothelial cells respond to fimbriae by secreting IL-8, which may be important in signalling neutrophil chemotaxis (Nassar et al. 2002). Epithelial cells and monocytes respond to fimbriae-mediated activation of TLR-2 by secreting cytokines (Eskan et al. 2007). This pathway may be particularly important in activating IL-12 secretion with the consequent activation of $CD4^+$ Th1 cells and NK cells. Innate signalling of adaptive immunity is also promoted by FimA-mediated uptake of *P. gingivalis* into DCs with consequent upregulation of IL-1β, TNF-α, IL-6 and IL-10 secretion by these cells (Jotwani & Cutler 2004).

Pathways activated by engagement of PRRs are integrated with those activated by independent extracellular signals. An example of this is the finding that *P. gingivalis* binding to the complement receptor CR3 suppresses TLR-2-induced IL-12 secretion from macrophages with consequential effects on *P. gingivalis* virulence in an animal model (Hajishengallis et al. 2007, 2008). Although this illustrates an important principle, our understanding of the integration of signals from multiple MAMPs and endogenous signalling molecules such as cytokines is in its infancy. The gingipains of *P. gingivalis* can also stimulate cytokine secretion via activation of protease-activated receptors (PARs). RgpB activates two different PARs (PAR-1 and PAR-2), thereby stimulating IL-6 secretion in epithelial cells (Lourbakos et al. 2001). Both Rgp and Kgp gingipains stimulate IL-6 and IL-8 secretion by monocytes via activation of PAR-1, PAR-2 and PAR-3 (Uehara et al. 2008). The RgpA–Kgp complex of *P. gingivalis* penetrates the gingival connective tissue and stimulates inter-cellular adhesion molecule-1 (ICAM-1), IL-8, IL-6 and monocyte chemoattractant protein-1 (MCP-1) in cultured human epithelial and fibroblast cells, although a reduction in these mediators was observed at high concentrations, suggesting that close to the plaque, where RgpA–Kgp complex concentrations are high, the secretion of inflammatory mediators is attenuated, whereas distal to the plaque, it is stimulated (O'Brien-Simpson et al. 2009). It has also been shown that proteases produced by *P. gingivalis*, particularly lysine gingipain, can subvert the host pro-inflammatory response by direct cytokine degradation (Stathopoulou et al. 2009).

Bacterial nucleic acids also function as MAMPs. The hypomethylated CpG regions of bacterial DNA engage TLR-9 and stimulate cytokine responses. There are only limited data on the effect of DNA from periodontal bacteria on host responses, though it has been reported that *P. gingivalis* DNA stimulates IL-6 and TNF-α secretion in human gingival fibroblasts (HGFs) (Takeshita et al. 1999, Nonnenmacher et al. 2003). More recent data from animal studies suggest that *P. gingivalis* DNA may downregulate pathways which lead to Th1 and Th2 cytokine secretion (Taubman et al. 2007). Also, DNA from both *P. gingivalis* and *Tannerella forsythia* stimulates IL-1β, TNF-α and IL-6 secretion in monocytes (Sahingur et al. 2010).

HGFs are responsive to LPS, and constitutively express mRNA for a variety of TLRs and NLRs, stimulation of which leads to production of pro-inflammatory cytokines such as IL-6, IL-8 and MCP-1 (Uehara & Takada 2007). HGFs challenged with *P. gingivalis* and *E. coli* LPS secrete IL-6 and IL-8 with no evidence of LPS tolerance, indicating that HGFs can sustain the inflammatory response in the periodontium (Ara et al. 2009). In addition to LPS, HGFs also respond to outer-membrane protein and polysaccharide of *P. gingivalis* by producing inflammatory cytokines (Imatani et al. 2001). HGFs from periodontally diseased tissue produced higher quantities of IL-1 before and after stimulation with *P. gingivalis* LPS compared with HGFs from healthy tissue, and pre-treatment of the cells with IL-1α enhanced the production of IL-6 (Kent et al. 1999). Primary HGFs and PDL fibroblasts respond to *P. gingivalis* by increasing gene expression for IL-1β, IL-6, IL-8, TNF-α and regulated on activation normal T-cell expressed and secreted (RANTES), with heterogeneity in responsiveness between fibroblasts from different donors, and this may be important in determining susceptibility to periodontitis (Scheres et al. 2010). Elevated levels of cytokines can also locally amplify responses to LPS. For example, $CD14^+$ HGFs that were primed with IFN-γ increased production of IL-8 in response to LPS through augmentation of the CD14–TLR system (Tamai et al. 2002).

HGFs respond to virulence factors from *Aggregatibacter actinomycetemcomitans* resulting in inflammatory cytokine secretion (Agarwal et al. 1995, Belibasakis et al. 2005), and both *A. actinomycetemcomitans* and *P. gingivalis* LPS were shown to augment osteoprotegerin (OPG) expression in HGFs (Kiji et al. 2007). HGFs have been shown to respond to both *A. actinomycetemcomitans* and *Campylobacter rectus* stimulation by production of IL-6 and IL-8 (Dongari-Bagtzoglou & Ebersole 1996), and evidence has been demonstrated for subpopulations of

fibroblasts in periodontitis patients that have higher cytokine secretory capacity compared with healthy controls (Dongari-Bagtzoglou & Ebersole 1998). Cell surface expression of ICAM-1 is upregulated in cultured HGFs in response to *P. gingivalis* and *Prevotella intermedia* as well as IL-1β, TNF-α and IFN-γ, and gingival tissues from patients with periodontitis had increased expression of ICAM-1 compared with healthy controls (Hayashi et al. 1994). IFN-γ, but not IL-1β or TNF-α, was shown to enhance expression of CD14/MyD88 on HGFs and their subsequent responsiveness to *A. actinomycetemcomitans* LPS as measured by IL-6 and IL-8 production (Mochizuki et al. 2004). Human PDL fibroblasts also respond to stimulation with *P. gingivalis* or *P. intermedia* by increasing mRNA expression of IL-1β, IL-6, IL-8, TNF-α, receptor activator of NF-κB ligand (RANKL) and OPG (Yamamoto et al. 2006). *E. coli* LPS has also been shown to stimulate both OPG and RANKL expression in human PDL fibroblasts by upregulating IL-1β and TNF-α (Wada et al. 2004).

Epithelial cells respond to bacterial stimulation, but it must be noted that most research has focussed on individual bacterial species as opposed to examining the effects of the biofilm. *Treponema denticola* failed to induce IL-8 production by primary gingival epithelial cells and this lack of epithelial cell response was suggested to potentially contribute to periodontal pathogenesis by a resulting failure to trigger neutrophil chemotaxis (Brissette et al. 2008). Human gingival epithelial cells challenged with *A. actinomycetemcomitans* increased expression of IL-1β and IL-8 mRNA (Uchida et al. 2001). IFN-γ was shown to prime oral epithelial cells to produce IL-8 and granulocyte macrophage colony-stimulating factor (GM-CSF) by upregulation of TLR-2, TLR-4, MD-2 and MyD88 mRNA expression following stimulation with LPS, lipoteichoic acid and peptidoglycan (Uehara et al. 2002). Human gingival epithelial cells challenged with live *P. gingivalis* produced high levels of IL-1β, whereas those challenged with *A. actinomycetemcomitans* produced high levels of IL-8, and *Fusobacterium nucleatum* induced the highest levels of pro-inflammatory cytokines (Stathopoulou et al. 2010). Human endothelial cells were also shown to respond to *P. gingivalis* by stimulating OPG via an NF-κB-dependent pathway, and thus could act as a source of OPG in periodontitis (Kobayashi-Sakamoto et al. 2004). Human umbilical vein endothelial cells produced the chemokines IL-8 and MCP-1 in response to stimulation with *P. gingivalis*, suggesting that endothelial cells might be involved in the accumulation and activation of neutrophils and monocytes at an early stage in periodontal pathogenesis (Mao et al. 2002).

Mononuclear cells are responsive to stimulation by periodontal pathogens. *P. gingivalis* and *F. nucleatum* were both shown to stimulate higher levels of IL-1 and IL-6 production by gingival mononuclear cells compared with PBMCs (Gemmell & Seymour 1993). *P. gingivalis* LPS has also been shown to enhance IL-1β and IL-18 expression in human monocytic cells (monomac-6 cells) (Hamedi et al. 2009). Human monocyte U937 cells that were differentiated into macrophages responded to *F. nucleatum* LPS by increasing secretion of IL-1β, IL-6, TNF-α and MMP-9 (Grenier & Grignon 2006). LPS from *P. intermedia* induced TNF-α mRNA expression and protein release in THP-1 monocyte-derived macrophages via MAPK signalling pathways (Kim et al. 2007). Cultured PBMCs from patients with periodontitis produced significantly more IL-1β than PBMCs from periodontally healthy controls, whether challenged with *A. actinomycetemcomitans* LPS or not (McFarlane et al. 1990). DCs also respond to bacterial products. For example, $CD83^+$ mature DCs infiltrate the lamina propria in human periodontitis tissues, and in vitro, monocyte-derived DCs pulsed with *P. gingivalis* strain 381 or its LPS underwent maturation and produced IL-1β, PGE_2, IL-10 and IL-12 (Jotwani et al. 2001). These findings suggest a role for DCs in the pathophysiology of periodontitis involving the activation and maturation of DCs induced by *P. gingivalis*.

We are also accumulating information about the interface between other bacterial components and host cells. For example, *A. actinomycetemcomitans* leucotoxin A (LTxA) is predominantly responsible for IL-1 secretion stimulated by this species (Kelk et al. 2008). *T. denticola* major outer sheath protein (msp) signals via TLR-2 (Nussbaum et al. 2009). *T. denticola* has a lipooligosaccharide which induces cytokine secretion in HGFs (Tanabe et al. 2008) and also produces a peptidoglycan which stimulates cytokine secretion (Tanabe et al. 2009). Bacteroides-specific protein A (BspA) from *T. forsythia* stimulates cytokine secretion in monocytes (Hajishengallis et al. 2002) and IL-8 secretion in gingival epithelial cells via TLR-2 signalling (Onishi et al. 2008).

Collectively, these studies reveal the complexity of biofilm signalling of cytokine responses, and although many signalling pathways mediate the interface between periodontal bacteria and the host, we have no real understanding of how these relate to the overall host response and what their relative importance is in periodontal pathogenesis. More research is needed to investigate the totality of cytokine responses as induced by the subgingival plaque biofilm.

Cytokines are key mediators of immune responses and drive tissue destruction

Cytokines drive the tissue destruction that results in the clinical manifestations of periodontitis through myriad overlapping effects on cells and mediators in the periodontium. The complex interactions between cytokines and immune responses make it difficult to distinguish and compartmentalize different aspects of the role of cytokines in driving tissue destruction. Indeed, it is somewhat artificial to try to do so, and it must be remembered that immune responses do not occur in isolation from each other. Because most research papers have limited themselves to very specific research questions (Table S1), however, then a compartmentalized approach is somewhat inevitable when reviewing the literature. This section will consider the role of cytokines in driving immune responses that lead to tissue destruction.

Cytokines drive the secretion of inflammatory mediators and destructive enzymes

Cytokines upregulate the production of inflammatory mediators in the periodontium (e.g. prostaglandins, MMPs, cytokines, chemokines) leading to tissue destruction. Multiple feedback loops develop; for example, cytokines induce the secretion of prostaglandins, and increased prostaglandin concentrations result in increased cytokine secretion (Noguchi et al. 2007). IL-1β and TNF-α induce COX-2 in oral epithelial cells (Zhang et al. 2003), and IL-1β upregulates COX-2 expression in HGFs (Morton & Dongari-Bagtzoglou 2001). IL-1β and TNF-α synergistically increase PGE_2

production in HGFs (Yucel-Lindberg et al. 1999) and TNF-α was shown to upregulate PGE_2 and COX production in HGFs via the JNK and NF-κB signalling pathways (Nakao et al. 2002, Bage et al. 2010). IL-1β was also shown to increase PGE_2 secretion by HGFs, and the combination of IL-1β and PGE_2 resulted in a synergistic increase in IL-6 secretion by these cells (Czuszak et al. 1996).

Numerous studies have identified that cytokines induce the secretion of other cytokines. IL-1β induces the expression of IL-6, IL-8 and TNF-α in HGFs, and also acts in an autocrine manner to induce further IL-1β expression (Agarwal et al. 1995, Chae et al. 2005). In a study of cytokine expression in HGFs obtained from non-inflamed gingiva, there was a dose-dependent stimulation of IL-6 and LIF mRNA and protein by IL-1β and TNF-α, and dose-dependent stimulation of IL-11 mRNA and protein by IL-1β (Palmqvist et al. 2008). In another study, TNF-α, IL-1β and PGF2α all stimulated IL-6 production in cultured HGFs, and PGF2α synergistically increased IL-6 production stimulated by TNF-α and IL-1β (Noguchi et al. 2001). TNF-α also induced IL-1α and IL-1β production in HGFs, and this was synergistically enhanced by the presence of bradykinin (Yucel-Lindberg et al. 1995). It was observed that the upregulation of IL-6 production by HGFs that is induced by IL-1β is mediated by the p38 MAPK and NF-κB signalling pathways (Chae et al. 2005). IL-1β and TNF-α act synergistically in stimulating IL-6 secretion by HGFs and this combination of cytokines was shown to be many hundreds of times more potent in stimulating IL-6 production than LPS (Kent et al. 1998). In a study of IL-1R2 expression in HGFs, over-expression of IL-1R2 by gene transfer downregulated expression of IL-1β mRNA and IL-6 mRNA in response to IL-1β stimulation (IL-8 mRNA expression was unaffected) (Chou et al. 2000). Cultured gingival epithelial cells stimulated with TLR-2 and TLR-5 ligands produced both IL-1β and TNF-α, and this was enhanced by the addition of IL-17 (Beklen et al. 2009). IL-1β and TNF-α induced IL-1α secretion in HGFs, and this production was differentially modulated by T-cell-derived cytokines including IFN-γ and IL-4 (Kobayashi et al. 1999). IL-10 was shown to inhibit *P. gingivalis* LPS-induced IL-6 secretion in HGFs (Wang et al. 1999).

Collectively, the above studies give an indication of the complexities of cytokine interactions in the periodontium and demonstrate that cytokines influence the secretion of other cytokines. This is confirmed by gene expression studies, for example, in a quantitative real-time PCR study of gingival biopsies, periodontitis patients had higher expression of TNF-α, MMPs, RANKL and OPG than controls (Garlet et al. 2004). A study of the ''gingival transcriptome'' in gingival biopsies collected in a human experimental gingivitis model revealed that during inflammation, the dominant expression pathway was the immune response, including upregulation of IL-1α, IL-1β, IL-8, RANTES, CSF3 and superoxide dismutase (Offenbacher et al. 2009). These (and other) genes exhibited reversed expression patterns on resolution of inflammation, implying that they are important in maintaining homeostasis in gingival inflammation. The central role of IL-1β in many aspects of periodontal homeostasis was confirmed by research showing that IL-1β induced differential expression of genes involved in cell stress, DNA repair, cell cycle and proliferation, angiogenesis and extracellular matrix turnover in human gingival keratinocytes (Steinberg et al. 2006). Recent research has again confirmed the huge impact of IL-1β on inflammatory networks in the periodontium, and 254 genes were found to be differentially expressed in IL-1β-stimulated HGFs (215 upregulated, 39 downregulated), with upregulated genes including inflammatory cytokines, NF-κB pathway members, chemokines, transcription factors, MMPS and adhesion molecules (Vardar-Sengul et al. 2009). Another real-time RT-PCR investigation of mRNA expression in tissue biopsies from chronic periodontitis patients found that, in active periodontal sites, RANKL, IL-17, IL-1β and IFN-γ were significantly over-expressed compared with inactive lesions, with a positive correlation between RANKL and IL-17 (Dutzan et al. 2009). A further multiplex analysis of GCF cytokine levels in patients with periodontitis undergoing treatment revealed that post-therapy, the levels of IL-1α, IL-1β, IL-2, IL-3, IL-6, IL-7, IL-8, IL-12p40, CCL5/RANTES, MCP-1, macrophage inflammatory protein-1α (MIP-1α) and IFN-γ at diseased sites all decreased (Thunell et al. 2010).

MMP production is also upregulated by cytokines (Cox et al. 2006). For example, IL-1β and TNF-α enhance MMP-1 and MMP-3 production in HGFs (Domeij et al. 2002). In a study of gingival tissues from periodontitis and healthy patients, IL-1β, TNF-α and IL-17 were elevated in periodontitis patients, and these cytokines (particularly IL-1β and TNF-α) induced pro-MMP-1 and MMP-3 in gingival fibroblasts (Beklen et al. 2007). In a study of human PDL cells derived from a primary cell line, IL-1β produced a sevenfold elevation in MMP-1 mRNA levels (Hoang et al. 1997). Similarly, in human PDL cells, IL-1β resulted in a dose-dependent increase in MMP-3 expression at both the mRNA and protein levels (Nakaya et al. 1997). Co-culture of fibroblasts and macrophages led to increased MMP-1 expression by macrophages and this was enhanced by high glucose levels (Sundararaj et al. 2009). In these studies, IL-6 released by fibroblasts was essential for augmentation of MMP-1 expression by the macrophages, and high glucose, IL-6 and LPS had a synergistic effect on MMP-1 expression. In another study, IL-6 was shown to result in a dose-dependent increase in MMP-1 expression by HGFs, but only when its soluble receptor, sIL-6R, was present (Irwin et al. 2002). IL-6 has also been shown to be a powerful stimulator of MMP-1 expression in U937 macrophages, and high glucose concentrations, LPS and IL-6 all act in concert to further increase MMP-1 release (Li et al. 2010).

Chemokine expression is stimulated by cytokines; for example, IL-1β and TNF-α increase the production of RANTES/CCL5 in HGFs (Mustafa et al. 2001) and IFN-γ, TNF-α and IL-4 cooperatively regulate CXCR3-agonistic chemokines in oral keratinocytes and fibroblasts (Ohta et al. 2008). Chemokines are produced by a wide variety of infiltrating and resident cells in the periodontium and play a key role in inflammation by influencing the distribution of leucocyte subsets in the tissues. In explanted HGFs from clinically healthy tissues, IL-1β, TNF-α and *E. coli* LPS all significantly increased production of CCL20, with synergism between IL-1β and TNF-α, whereas IFN-γ decreased IL-1β-induced CCL20 production (Hosokawa et al. 2005a). In a further study, HGFs were exposed to pro-inflammatory cytokines (IL-1β, TNF-α), a Th1 cytokine (IFN-γ), Th2

cytokines (IL-4, IL-13), Th17 cytokines (IL-17A, IL-22) and Treg cytokines (IL-10, TGF-β1) (Hosokawa et al. 2009a). The HGFs produced CXCL10 following stimulation with IL-1β, TNF-α and IFN-γ. Treatment with IFN-γ in combination with IL-1β or TNF-α resulted in synergistically increased production of CXCL10, whereas IL-4, IL-13 and IL-10 inhibited CXCL10 production by IFN-γ- or TNF-α-stimulated cells. This research group has conducted a number of studies evaluating the impact of cytokines on chemokine secretion in HGFs, and has found that HGFs increased CXCL12 secretion following stimulation with TNF-α, IFN-γ, TGF-β, RANTES and MIP-3α (Hosokawa et al. 2005b). Further, TNF-α and IFN-γ enhanced ICAM-1 and vascular cell adhesion molecule 1 expression in HGFs, and IL-1β upregulated ICAM-1 expression (Hosokawa et al. 2006). IL-1β, TNF-α and IFN-γ also increased expression of CXCL16 in HGFs with synergism between IFN-γ and IL-1β (Hosokawa et al. 2007), and HGFs also expressed CXCR6, the receptor for CXCL16 (Hosokawa et al. 2009b). A combination of TNF-α and IL-4/IL-13 increased CCL17 expression (Hosokawa et al. 2008). The same researchers investigated the effects of TNF ligand superfamily member 14 (TNFSF14) on IFN-γ-induced CXCL10 and CXCL11 production in HGFs and showed that TNFSF14 enhanced IFN-γ-induced secretion of CXCL10 and CXCL11 (Hosokawa et al. 2010). Synergism between cytokines in inducing chemokine release was shown in a pre-osteoblast cell line in which IL-17 and TNF-α cooperatively induced the LPS-inducible CXC chemokine LIX at the mRNA and protein level (Ruddy et al. 2004), and also in HGFs in which the combination of IL-1β and TNF-α synergistically increased IL-8 secretion, an effect that was suppressed by IFN-γ (Takigawa et al. 1994).

Studies of chemokine expression in gingival biopsies identified that MIP-1α and IFN-γ-inducible protein 10 and their receptors were more prevalent in aggressive periodontitis and associated with higher IFN-γ and lower IL-10 expression, whereas in chronic periodontitis, there was higher expression of MCP-1 and IL-10 (Garlet et al. 2003). MIP-1α expression is also induced in epithelial cells and polymorphonuclear leucocytes (PMNLs) by IL-1β, *P. gingivalis* and *A. actinomycetemcomitans* LPS (Ryu et al. 2007). Chemokine expression in the gingival tissues has been investigated (Gemmell & Seymour 1998), and it is clear from the above studies that chemokines are involved in periodontal pathogenesis, driving migration and recruitment of cells such as PMNLs, DCs, NK cells, macrophages and lymphocyte in the tissues, although the complexities of the identified pathways and the heterogeneous nature of the research studies to date ensure that we still do not have a holistic understanding of the role of chemokines in periodontitis.

Cytokines have multiple effects on resident and infiltrating cells

In addition to the effects of cytokines on mediator synthesis and release as described above, cytokines have multiple effects on cells in the periodontium that drive tissue destruction. For example, enhanced accumulation of PMNLs has been reported in the gingival tissues of patients with periodontitis, and has been associated with upregulated IL-8, ICAM-1, IL-1β and TNF-α expression (Liu et al. 2001). In general, researchers have focussed in particular on the role of HGFs and lymphocytes in the periodontium and their responsiveness to cytokines.

IL-1β stimulates IL-6, IL-8, PGE_2 and MMP-1 secretion in HGFs via activation of MAPK/AP-1 and NF-κB (Kida et al. 2005). The complexities of fibroblast responses to cytokines are exemplified by research, which showed that HGFs responded to IL-1α stimulation by increasing expression of OPG mRNA whereas in PDL fibroblasts, RANKL mRNA expression was increased, suggesting possible differential roles for gingival HGFs compared with PDL fibroblasts (Hormdee et al. 2005). Similarly, HGFs produced significantly more MIP-1α, stromal cell-derived factor-1 (SDF-1) and IL-6 when challenged with *P. gingivalis* LPS compared with PDL fibroblasts cultured from biopsies taken from the same donors, supporting that fibroblasts from different tissue compartments differentially contribute to the balance of cytokines in the periodontium (Morandini et al. 2010). The impact of combinations of cytokines on fibroblast responses has also been investigated. For example, when administered separately, IL-17-induced IL-8 expression by HGFs and had minimal impact on ICAM-1, whereas IFN-γ augmented the expression of HLA-DR and ICAM-1 but not IL-8 (Mahanonda et al. 2008). When IL-17 and IFN-γ were combined, there was marked enhancement of IL-8 and ICAM-1 expression. The same research group identified that TNF-α enhanced TLR ligand-induced IL-8 production by HGFs, whereas IFN-γ enhanced IDO (indoleamine 2,3-dioxygenase) expression (Mahanonda et al. 2007). Interactions between cells also add complexity to our understanding of the role of cytokines in driving cellular responses. For example, co-culture of HGFs with human lymphoid cells resulted in increased expression of IL-1α, IL-1β and IL-6 mRNA in the HGFs (Murakami et al. 1999). In addition, IL-1β mRNA expression was synergistically increased when the HGFs directly interacted with lymphoid cells in the presence of exogenous IL-1β. It is clear, therefore, that interactions between cells transduce activation signals in HGFs that result in increased inflammatory cytokine mRNA expression, and that this is amplified by the presence of cytokines in the local environment.

Cytokines also drive tissue destruction through their impact on lymphocytes. In terms of the contribution of adaptive immune responses to soft-tissue breakdown, it is clear that B lymphocytes and plasma cells dominate among cells in the periodontium. T-cells are the main regulatory cells, but B-cells also play a role in periodontal pathogenesis. B-cells are activated by cytokines such as IL-1β and TNF-α; for example, it has been shown that IL-1α and IL-1β promote IgG2 production (Ishihara et al. 2001) and IL-6 upregulates IL-7 production by B-cells (Colucci et al. 2005). In studies of cultured PBMCs from *A. actinomycetemcomitans* seropositive patients with localized aggressive periodontitis, IL-1α, IL-1β, IFN-γ, IL-12 and PGE_2 were all necessary for optimal production of anti-*A. actinomycetemcomitans* IgG (Tanaka et al. 2006). B-cells can act as APCs, and express class II antigens and contribute to antigen presentation (Berglundh et al. 2007). After culture with either *A. actinomycetemcomitans* or *P. gingivalis*, activated B-cells performed as potent APCs in mixed leucocyte reactions, stimulating T-cells to produce high levels of IFN-γ suggesting that B-cells which infiltrate the gingival tissues play a role as APCs in the regulation of local T-cell responses

(Mahanonda et al. 2002). Activated B-cells can be found in the periodontal tissues and IL-6 and IL-10 concentrations were also significantly elevated in inflamed gingival tissues (Aramaki et al. 1998). IL-10 generally functions as a regulatory cytokine but does also exhibit other activities such as activation of B-cells (Mocellin et al. 2004). For example, when PBMCs were cultured in the presence of *P. gingivalis* and IL-10, significantly increased B-cell proliferation was observed compared to when cells were cultured with *P. gingivalis* alone (Champaiboon et al. 2000). It is likely, therefore, that IL-10 stimulates B-cell activity in the periodontium (Berglundh et al. 2007), although this remains to be confirmed. The different aspects of IL-10 biology (immunosuppressive *versus* immunostimulatory) likely depend on the experimental conditions and the local cytokine environment. An emerging concept is that in infections of low/moderate virulence, IL-10 from DCs and macrophages drives IL-10 production by Treg cells, which limits pathology but permits escape of pathogens from immune control, leading to persistent infection. On the other hand, in highly virulent infections in which there is a strong pro-inflammatory response, IL-10 produced from large numbers of induced Treg cells appears to be necessary to minimize pathology during resolution of the infection. IL-10 is therefore of benefit to the host (by limiting pathology) and also the pathogen (by permitting persistent infection) (Couper et al. 2008). Treg cells, an important source of IL-10, have been identified in inflamed periodontal tissues, with increased expression of TGF-β and IL-10 in periodontitis compared with gingivitis, suggesting that these cells may play a regulatory role in periodontal diseases (Nakajima et al. 2005).

Activation of B-cells during antigen-specific immune responses leads to differentiation into antibody-producing plasma cells. Plasma cells also produce cytokines including TNF-α, IL-6, IL-10 and TGF-β and thus contribute to tissue breakdown. In a co-culture model of *P. gingivalis* LPS-stimulated monocytes and T-cells, it was found that IFN-γ enhanced LPS-stimulated IL-12 secretion in monocytes, and IL-12 enhanced LPS-stimulated IFN-γ secretion by T-cells; this suggested an ''activation loop'' involving these cytokines which was influenced by periodontal bacteria (Yun et al. 2002). These findings underscore the importance of lymphocyte subpopulations as well as the nature of the local cytokine milieu in the development of periodontitis.

Cytokines drive bone resorption

Alveolar bone resorption occurs when the levels of inflammatory mediators in the overlying soft tissues reach a certain threshold at a critical distance from the bone surface and activate pathways leading to bone resorption (Graves & Cochran 2003). Infiltrating cells such as macrophages and lymphocytes as well as resident cells (fibroblasts, tissue macrophages) secrete a wide variety of cytokines and mediators such as IL-1β, TNF-α, IL-6, IL-11, IL-17 and PGE_2 which can regulate osteoclastic activity via RANK/RANKL/OPG, or can contribute to bone loss via RANK-independent pathways (Teng 2006). The latter include the ability of IL-1β and TNF-α to activate osteoclasts independently of RANK via IL-1R1 and TNFR1 leading to differentiation and activation of osteoclasts via NF-κB, with coupling of TNFR1 and RANK signalling pathways (Zhang et al. 2001). IL-1β is a potent stimulator of bone resorption and plays a role in the multiple steps of osteoclast differentiation, multi-nucleation, activation and survival (Nakamura & Jimi 2006). Inhibition of IL-1β and TNF-α either systemically or locally has been shown to inhibit alveolar bone loss in animal models of periodontitis (Assuma et al. 1998, Graves et al. 1998, Delima et al. 2001).

RANK/RANKL/OPG play a critical role in regulating bone metabolism, and function in a network that is essential for controlling osteoclast development and function (Koide et al. 2010). Many cytokines lead to increased RANKL expression and RANKL is increased in inflamed periodontal tissues (Crotti et al. 2003). For example, IL-1β stimulates PGE_2 release from fibroblasts and osteoblasts, which in turn stimulates bone resorption via increased RANKL expression (Akaogi et al. 2006). IL-6, IL-11, LIF and oncostatin M stimulate osteoblast formation and bone resorption via upregulation of RANKL (Palmqvist et al. 2002, Ochi et al. 2007). In a mouse model, inflammatory mediators that induced bone resorption (IL-1β, IL-6, IL-11, IL-17, TNF-α) increased the expression of RANKL and decreased the expression of OPG, whereas those that inhibited osteoclastogenesis (IL-13, IFN-γ, TGF-β1) suppressed RANKL expression and/or increased OPG expression (Nakashima et al. 2000). It has also been reported (again, in a mouse model) that patterns of cytokines in the periodontal tissues determine the balance between MMPs/TIMPs and RANKL/OPG (Garlet et al. 2006). Increased gingival RANKL:OPG ratios in patients with periodontitis compared with healthy patients are a consistent finding in clinical studies (Liu et al. 2003, Cochran 2008). In an analysis of gingival tissue homogenates from patients with healthy gingiva or periodontitis, concentrations of RANKL and IL-10 were negatively correlated whereas RANKL and IL-1β were positively correlated, and IL-10 suppressed both soluble RANKL (sRANKL) and membrane RANKL expression by PBMCs stimulated with *A. actinomycetemcomitans* (Ernst et al. 2007).

Activated T and B lymphocytes in the gingival tissues of patients with periodontitis express RANKL (Vernal et al. 2006, Han et al. 2009). Indeed, these cells have been reported to be the primary source of RANKL in alveolar bone resorption lesions, with $>50\%$ of T-cells and $>90\%$ of B-cells expressing RANKL (Kawai et al. 2006). This finding underpins the importance of the links between adaptive and innate immunity in periodontal tissue breakdown. B-cells mediate bone resorption and immunoglobulin-producing $CD20^+$ B-cells can contribute to alveolar bone loss by expression of RANKL (Han et al. 2006, 2009, Kawai et al. 2006). In another study, osteoclast precursor cells were co-cultured with activated B or T lymphocytes ($CD4^+$ and $CD8^+$) in the presence of M-CSF alone or M-CSF plus sRANKL (Choi et al. 2001). The activated B-cells and $CD4^+$ (but not $CD8^+$) cells induced osteoclast differentiation in the presence of M-CSF alone. In the presence of M-CSF and sRANKL, B-cells induced the formation of osteoclasts and increased bone resorption whereas the $CD8^+$ T-cells suppressed osteoclastogenesis.

The majority of RANKL produced by T-cells is the soluble form, sRANKL (Kanamaru et al. 2004). T-cells from PBMCs of periodontitis patients were shown to over-express RANKL and TNF-α, leading to spontaneous osteoclastogenesis (Brunetti et al. 2005). Th1 and Th17 lymphocytes play a key role in inflammation-induced bone resorption (Sato et al. 2006, Stashenko et al.

2007), and IL-17 contributes to increased RANKL expression in osteoblasts in synovial fluid (Kotake et al. 1999). IL-17 likely plays a role in driving alveolar bone loss in periodontitis (Oda et al. 2003, Cardoso et al. 2008, 2009) and also in recruiting neutrophils to the periodontal tissues (Yu et al. 2007). IL-17 therefore appears to be important in both the pathogenesis of inflammation-driven bone resorption as well as having a protective effect mediated by neutrophil trafficking (Yu et al. 2007). IFN-γ produced by T-cells has been shown to suppress osteoclastogenesis by promoting TNF receptor-associated factor 6 degradation, thereby resulting in inhibition of RANKL-induced activation of NF-κB and JNK (Takayanagi et al. 2000). This provides evidence of cross-talk between the TNF and IFN cytokine families, and supports the concept that activated T-cells contribute to maintaining bone homeostasis through a controlling effect on RANKL activity in addition to their pro-resorption activities via expression of RANKL and cytokine secretion. TGF-β can also inhibit osteoclastogenesis, depending on the presence of osteoblasts, possibly by upregulating OPG production and thereby inhibiting RANKL/RANK signalling in osteoclasts and their precursors (Takai et al. 1998, Yan et al. 2001). On the other hand, in the absence of osteoblasts or stromal cells and in the presence of lymphocytes, TGF-β promotes osteoclastogenesis (Kaneda et al. 2000, Massey et al. 2001). Clearly, more research is required in the context of human periodontitis, but the evidence supports a major role for activated T lymphocytes in mediating bone resorption and the adaptive immune response.

DCs, the most potent APCs which are responsible for activation of naïve T-cells are also likely to play a direct role in bone osteoimmunology. Evidence supports that DCs may act as osteoclast precursors that can develop into DC-derived osteoclasts under inflammatory conditions (Alnaeeli et al. 2007), particularly the presence of M-CSF and RANKL as part of innate immune responses, and also following stimulation from RANKL-expressing T-cells during adaptive immunity (Cutler & Teng 2007). RANK–RANKL signalling in DCs can also enhance DC survival, promote antigen-presenting functions (Cutler & Teng 2007) and enhance interactions with T-cells (Page & Miossec 2005). Resident cells in the periodontium (e.g. PDL fibroblasts) can also be induced to express RANKL/OPG (Hasegawa et al. 2002, Nagasawa et al. 2002) and it is clear that regulation of RANK/RANKL/OPG is more complex than previously thought. The term ''RACIN'' has been coined to describe the ''RANKL and Cytokine Interactions Network'' (Teng 2006), a suggested complex cytokine network that regulates bone resorption.

Summary

Cytokines have broad molecular interactions that are relevant to many aspects of immunity and inflammation. It is clear that in periodontal pathogenesis, cytokines have wide ranging and overlapping functions, as would be expected in any tissue compartment exposed to a chronic bacterial challenge and in which there is persistent chronic inflammation. In simple terms, the balance between pro- and anti-inflammatory cytokines and regulation of their receptors and signalling pathways determines the extent of periodontal tissue destruction. It is becoming increasingly clear that cytokines interact and function in networks, but we do not fully understand the complexities of these networks.

To date, periodontal research has not set out to characterize cytokine networks in the periodontium. A biological network comprises a framework for the interaction of the constituent elements and can be defined based on experimental data and modelling using bioinformatics. An important property of such networks is that changes in the levels of individual elements may not have predictable effects on function, indeed there may be new emergent properties not apparent by the investigation of individual elements (Gardy et al. 2009). Although it has long been recognized that cytokines are one example of a biological network (Balkwill & Burke 1989, Nathan & Sporn 1991), we have very limited understanding of how cytokine networks might function in human disease. A major challenge is to characterize the totality of the cytokine response, the consequences for the host immune response, and what contribution this makes to disease pathogenesis. Fortunately, we now have the tools to examine cytokine responses more holistically than before. For example, microarray technology to analyse gene expression patterns in cells and tissues on a genome-wide basis is beginning to be used by periodontal researchers (Mans et al. 2010, Taylor 2010). The studies fall into two main categories: in vivo studies of gene expression in periodontal tissues and cells from patients (Papapanou et al. 2004, 2007, Demmer et al. 2008) and in vitro studies of cell lines exposed to periodontal bacteria or their molecular components (Chen et al. 2007, Handfield et al. 2008). The strengths and weaknesses of these approaches and the challenges of analysing the large quantity of data generated by these studies have been reviewed elsewhere (Handfield et al. 2008, Mans et al. 2010). In general terms, both in vitro and in vivo analyses confirm that upregulation of genes involved in immune responses (including cytokines) is a consistent finding in periodontal inflammation (Demmer et al. 2008, Handfield et al. 2008).

It is difficult to draw all the separate threads of research together that have investigated cytokine and lymphocyte interactions in the periodontium, given the huge heterogeneity of the experimental systems that have been used. Nonetheless, it is clear that cytokine networks have evolved to protect the host via innate and adaptive immune responses. Imbalance in cytokine networks has been implicated in the pathogenesis of conditions such as type 2 diabetes (Arend et al. 2008, Schroder et al. 2010) and rheumatoid arthritis (McInnes & Liew 2005, McInnes & Schett 2007) and it is very likely that perturbations in cytokine networks determine the progression of periodontitis (Graves 2008). At present, the strongest evidence for cytokines functioning in networks in periodontal pathogenesis exists for IL-1β, TNF-α, IL-6 and RANK/RANKL/OPG, and ongoing research is elucidating the role of other cytokines and chemokines in periodontal inflammation. There is potential for extensive variation between individuals in periodontal inflammatory responses given the large number of mediators involved, the multiple and overlapping functional links between cytokines and lymphocytes, and the many opportunities for control of activity (such as transcriptional regulation, decoy receptors, presence of activating enzymes, availability of accessory proteins and feedback loops).

In the context of periodontal pathogenesis, we can make the following conclusions:

- It seems that cytokines interact and function within networks, but we do not yet understand the networks, or how the balance of the networks relates to the clinical course of disease.
- Most published work on cytokine biology and immune responses in periodontitis has involved model systems which are far removed from the microanatomical complexity of the human periodontium and the many interacting elements of periodontal pathogenesis. Also, the widespread use of animal models can limit the transferability of knowledge to the human situation. To address these issues, we need to develop more meaningful in vivo and ex vivo models of periodontitis, which will give us better understanding of how the human host responds to a polymicrobial challenge.
- Much ''raw data'' exists on how individual cytokines interact in experimental systems; however, we are yet to describe the nature and boundaries of the ''cytokine network'' in periodontal pathogenesis. Cytokine biology has progressed from simple measurements of the levels of cytokines to investigations of the molecular pathways that link sensing of microbial infection to cytokine synthesis and regulation. We need to embrace modern bioinformatics approaches to gain insight into the holistic nature of immune responses in periodontitis, rather than focussing on simple interactions and studies of single mediators. As modern multiplex and genome-wide approaches reveal novel potential mediators of periodontal inflammation (e.g. adipokines, chemokines, novel cytokines), we need to adopt standardized methods for assessing their role in periodontitis.
- There is increasing appreciation of the diversity of lymphocyte responses and the role of cytokines in mediating their development and function. Research in these areas will need to be expanded if we are to understand the role of T- and B-cells in periodontal pathogenesis and improve our knowledge of the totality of immune responses in the periodontium.
- Unified themes need to be developed between different research groups throughout the world to tackle common research problems in periodontal pathogenesis, and to develop a cohesive strategy for investigating cytokine networks in driving immune responses and periodontal tissue breakdown. This will give us the best chance for developing improved therapeutic strategies for this common disease.

Note Added in Proof

IL-1F6, IL-1F8, IL-1F9 and IL-1F5 have recently been renamed IL-36α, IL-36β, IL-36γ and IL-36Ra, respectively, and IL-1F7 has been renamed IL-37 with the splice variants of this cytokine designated IL-37a–e (Dinarello et al. 2010, Nold et al. 2010).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Numbered list of the 259 references identified in the literature search (search strategy described in Table 2). References in bold have been cited in the text of the paper. Note the huge heterogeneity of the identified research.

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Clinical Relevance

Scientific rationale for the study: Periodontitis is a complex inflammatory disease, and technical advances have improved our ability to study the molecular and cellular activity that underpins the clinical changes observed in periodontitis. The purpose was to review evidence regarding a role for cytokine interactions and how they relate to lymphocyte function in periodontal pathogenesis.

Principal findings: Periodontal pathogenesis involves multiple interacting cytokines that likely function in networks that drive cellular activity, immune responses and tissue destruction associated with periodontitis. The strongest evidence for cytokine interactions in periodontal disease exists for pro-inflammatory mediators such as IL-1β and TNF-α, and there is emerging information on cytokines relevant to other aspects of periodontal pathogenesis such as T-cell regulation, bone cell activity and leucocyte chemotaxis. We do not yet fully understand how cytokine interactions impact on lymphocyte function and how this contributes to periodontal pathogenesis.

Practical implications: Improving our knowledge of cytokine interactions and immune responses in the periodontium is fundamental to understanding the clinical course of the disease and developing novel treatment strategies for periodontitis. Although we have much basic information, there is a need to develop consistent research methodologies and to utilize modern multiplex and bioinformatics approaches to improve our understanding further.