

Infection and inflammatory mechanisms

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Abstract

This introductory article examines the potential mechanisms that may play a role in the associations between periodontitis and the systemic conditions being considered in the EFP/AAP Workshop in Segovia, Spain. Three basic mechanisms have been postulated to play a role in these interactions; metastatic infections, inflammation and inflammatory injury, and adaptive immunity. The potential role of each alone and together is considered in in vitro and animal studies and in human studies when available. This is not a systematic or critical review, but rather an overview of the field to set the stage for the critical reviews in each of the working groups.

Key words: cardiovascular disease; infection; inflammation; pathogenesis; periodontitis; systemic diseases

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Two disorders/diseases can simultaneously occur or may develop sequentially where progression or exacerbation of one disease may affect the second disease. In parallel with Koch's postulates, which are used to identify the aetiological agents of an infectious disease, the criteria for a causal association between two diseases have been defined and are known as the Bradford Hill criteria. These include epidemiological association, biological plausibility and the impact of intervention of one on the second disease.

A large body of evidence exists relevant to the association of periodontitis with diabetes mellitus, cardiovascular disease and dental focal infections. Three mechanisms

have been postulated to play a role in non-oral manifestations of oral diseases (Thoden van Velzen et al. 1984): metastatic infections, dissemination of bacterial toxins and immunological injury. The word *metastasis* comes from the Greek "displacement"; *μετά*, *meta*, "next", and *στάσις*, *stasis*, "placement". *Metastasis*, or *metastatic disease*, has been defined as the spread of a disease from one organ or part of the body to another non-adjacent organ or body part. The definition is not limited by the common usage involving malignant tumour cells; infection and inflammation have the capacity to *metastasize* (Chiang & Massague 2008).

In the context of the relationship between periodontal disease and systemic diseases, the underlying assumption is that periodontitis is an infection that causes an inflammatory disease that metastasizes. This can be metastasis of the infection (bacteraemia and infection at non-oral sites caused by oral bacteria or other direct bacterial actions), inflammation and inflammatory mediators having an impact on systemic inflammation

mediated by innate immune cells and mediators, activation of adaptive immunity and the systemic consequences, or an undefined combination of any or all of these potential mechanisms. However, it is plausible, if unlikely based on available data, that the associations are the result of common risk factors and not causally related. From our understanding of the biology of the relationship between periodontitis and systemic disease, it remains clear that the relationship is not linear, but complex.

The purpose of this introductory Supplement article is to discuss the potential mechanisms underpinning the associations between periodontal disease and systemic conditions. This not intended to be a systematic or critical review, but than an overview of the possibilities based upon our understanding of the systemic consequences of periodontal infection and inflammation. The classification of Gonzalez-Periz et al. (2009), Kinane et al. (2005) has been modified for the purposes of this Supplement article, where metastatic infections and bacterial toxins will be considered

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under the heading of ‘Infection’ followed by ‘Inflammation’ and its consequences, and finally ‘Adaptive Immunity’.

Infection

Periodontal disease (gingivitis and periodontitis) is a destructive disease of the gingiva and of the supporting structures of the teeth, which develops through inflammatory processes induced by a microbial biofilm. This fundamental periodontal principle was first established by a landmark study by Loe et al. (1965). Periodontal bacteria possess a plethora of virulence factors that induce cells to produce inflammatory mediators at the gingival level. It is also important to note that inflammation is usually not confined only to periodontal tissues. Bacteria and inflammatory mediators may enter the blood and disseminate systemically having a measurable impact on systemic inflammation. The epidemiological evidence linking periodontitis to the progression of systemic diseases, such as cardiovascular disease, adverse pregnancy outcomes and diabetes mellitus, is associated with both bacteraemia and elevated levels of various markers of systemic inflammation. Periodontal disease is not universally expressed in people with poor oral hygiene that harbour periodontal pathogens; disease expression also requires a susceptible host (Offenbacher 1996). The determinants of susceptibility for destructive periodontal disease are not well defined.

In addition, virulence factors such as cytotoxins, proteases and haemagglutinins, structural molecules of the bacteria, including lipopolysaccharide (LPS) and peptidoglycan (PGN), interact with the host immune system. Most of these molecules have conserved motifs known as pathogen-associated molecular patterns (PAMPs), which are recognized by host cell receptors called pattern recognition receptors (PRRs). PRRs detect PAMPs in the environment and activate specific signalling pathways in host cells that initiate inflammatory responses. Bacterial virulence factors including PAMPs are LPS, PGN, lipoteichoic acid (LTA), fimbriae, proteases, heat-shock proteins (HSPs), formyl-methionyl-leucyl-phenylalanine

(fMLP) and toxins. PRRs include the Toll-like receptors (TLRs) and a variety of G-protein coupled receptors (GPCRs) (Madianos et al. 2005). However, it is important to note that most of these proposed interactions have only been observed in vitro or in animal models.

Metastatic infection

Metastatic or focal infection by bacterial translocation connotes an infectious disease mediated by microorganisms that have originated from a distant body site. In periodontitis, the infection is not limited to the gingiva or oral cavity. The resultant bacteraemia comes from perturbation of ulcerated periodontal tissues by simple acts of tooth brushing and eating disseminating whole bacteria and their products and toxins such as LPS (Kinane et al. 2005). Non-oral infections caused by oral pathogens were first described decades ago and include among others endocarditis, lung infections, and liver and brain abscesses (van Winkelhoff & Slots 1999). Oral bacteria, disseminated from periodontal, endodontic or mucosal lesions can survive in the blood stream and may adhere at non-oral body sites. A *locus minoris resistentiae* (such as scar tissue, or microangiopathy, prosthetic devices) may be a prerequisite for adherence of oral bacteria.

Using PCR, DNA of periodontal bacteria has been detected in carotid atheromas and other sites of pathology distal to the source (Haraszthy et al. 2000), but the role of these observations in the pathogenesis of vascular disease remains unclear. It is possible that these bacteria cause tissue damage or initiate inflammation. In certain instances, they grow in tissues causing disease as in the lung (Raghavendran et al. 2007), but bacteria identified in atheromas, for instance, do not form colonies. In a series of experiments using APO-E knockout mice, Genco and co-workers (Deshpande et al. 1998a,b) induced cardiovascular lesions with oral administration of *Porphyromonas gingivalis* and subsequently recovered the bacterium from fatty streaks in the aorta. These findings were not supported in rabbit experiments (Jain et al. 2003). The role of disseminating infection in each sys-

temic condition will be evaluated in the subsequent systematic and critical reviews.

Inflammation

Despite the localized nature of periodontal disease, infection of the sulcus/periodontal pocket can lead to inflammatory responses beyond the periodontium. Several biological pathways have been identified linking periodontal disease to induction of systemic inflammation. In health, the sulcular epithelium and local innate immunity act as a natural barrier that prevents bacterial penetration. In gingival health, only a small number of bacteria, mostly facultative anaerobes, are found in the gingival crevice and bloodstream. However, in periodontal disease, inflamed and ulcerated subgingival pocket epithelium is vulnerable to bacteria and provides a port of entry. Recently, Nesse et al. (2008) described a technique to calculate the amount of inflamed periodontal tissue (Periodontal Inflamed Surface Area, PISA) to assess the inflammatory burden posed by periodontitis. They found a dose-response relationship between PISA and HbA1c in type 2 diabetics.

Bacteraemia is further aggravated by mechanical means during tooth brushing, chewing, oral examination, endodontic treatment (Debelian et al. 1995) and scaling and root planing (Kinane et al. 2005). Microorganisms that gain access to the blood are usually eliminated by the reticuloendothelial system within minutes (transient bacteraemia) with no clinical symptoms (Li et al. 2000). However, it is plausible that bacteria persist at distal sites disseminating virulence factors that act as soluble antigens. Bacteria and bacterial antigens that are systemically dispersed trigger significant systemic inflammation. Leucocytes, endothelial cells and hepatocytes respond to bacteria/virulence factors with secretion of pro-inflammatory immune mediators [cytokines, chemokines, C-reactive protein (CRP)]. With continued exposure, soluble antigens react with circulating specific antibody to form immune complexes that further amplify inflammation at sites of deposition (Thoden van Velzen et al. 1984, Li et al. 2000). Likewise, pro-inflammatory mediators, such as

IL-1 β , IL-6, TNF- α and PGE₂, produced locally in the inflamed gingival tissues may “spill” into the circulation and have systemic impact, such as induction of endothelial dysfunction (Amar et al. 2003, Elter et al. 2006). Pro-inflammatory cytokines in circulation induce leucocytosis and acute-phase proteins. In addition to CRP, acute-phase reactants include serum amyloid A protein, fibrinogen, plasminogen activator inhibitor 1, complement proteins, LBP and soluble CD14; all are implicated in the systemic conditions linked to periodontitis.

Innate immunity – The inflammatory response

Cytokines are low molecular weight proteins that initiate and perpetuate inflammation, as well as regulate the amplitude and duration of the response. The genetic regulation leading to secretion of pro-inflammatory cytokines from a variety of cells is generally dependent on the activation of NF κ B nuclear protein activation of transcription (Baldwin 1996, Hanada & Yoshimura 2002). The NF κ B regulated pathways are activated by PAMPs such as LPS through the TLR pathway (Hanada & Yoshimura 2002).

Cytokines are produced by resident cells, such as epithelial cells and fibroblasts, and phagocytes (neutrophils and macrophages) in the acute phase and early chronic phase of inflammation, and by immune cells (lymphocytes) in adaptive immunity (Ara et al. 2009). After microbial recognition, cytokines of the innate response, including TNF- α , IL-1 β and IL-6, are the first secreted in periodontal disease pathogenesis (Garlet 2010). IL-1 β and IL-6 are signature innate cytokines and have been characteristically associated with inflammatory cell migration and osteoclastogenesis (Graves et al. 2008, Fonseca et al. 2009). TNF- α is a pleotropic cytokine that has many functions from cell migration to tissue destruction (Peschon et al. 1998, Dinarello 2000, Wajant et al. 2003, Kindle et al. 2006). TNF- α up-regulates the production IL-1 β and IL-6 (Okada et al. 1997, Dinarello 2000, Wajant et al. 2003, Kwan Tat et al. 2004, Garlet et al. 2007, Graves et al.

2008, Musacchio et al. 2009). TNF- α is also correlated with extracellular matrix degradation and bone resorption through actions promoting secretion of MMPs and RANKL (Graves & Cochran 2003, Garlet et al. 2004, Graves et al. 2008) and coupled bone formation (Behl et al. 2008). Accordingly, TNF α in circulation significantly impacts systemic inflammation and associated systemic conditions (CRP and CVD, obesity, Type 2 diabetes).

Chemokines are chemotactic cytokines that play a very important role in phagocytic cell migration to the site of infection. Once blood leucocytes exit a blood vessel, they are attracted by functional gradients of chemotactic factors to the site of infection (Rossi & Zlotnik 2000, Zlotnik & Yoshie 2000). Chemokines are synthesized by a variety of cells including endothelial, epithelial and stromal cells, as well as leucocytes. Functionally, chemokines can be grouped as homeostatic or inflammatory (Moser et al. 2004). In addition to their cell trafficking role, chemokines provide messages leading to other biological processes, such as angiogenesis, cell proliferation, apoptosis, tumour metastasis and host defence (Rossi & Zlotnik 2000, Zlotnik & Yoshie 2000, Moser et al. 2004, Rot & von Andrian 2004, Esche et al. 2005). Bacterial peptides are also chemotactic for inflammatory cells. Chemokines target leucocytes of the innate immune system, as well as lymphocytes of the adaptive immune system (Terricabras et al. 2004).

Lipid mediators of inflammation

Prostaglandins (PGs) are derived from hydrolysis of membrane phospholipids. Phospholipase A₂ cleaves the *sn*-2 position of membrane phospholipids to free arachidonic acid, a precursor of a group of small lipids known as eicosanoids (Lewis 1990). Arachidonic acid is metabolized by two major enzyme pathways. Lipoxigenases (LO) catalyse the formation of hydroxyeicosatetraenoic acids (HETEs) leading to the formation of leucotrienes (LT). Cyclooxygenases (COX-1 and COX-2) catalyse the conversion of arachidonic acid into prostaglandins,

prostacyclins and thromboxanes. Prostaglandins have 10 sub-classes, of which D, E, F G, H and I are the most important in inflammation (Gemmell et al. 1997). Inflamed gingiva synthesizes significantly larger amounts of prostaglandins when incubated with arachidonic acid than does healthy gingiva (Mendieta et al. 1985). Prostaglandin E₂ (PGE₂) is a potent stimulator of alveolar bone resorption (Goodson et al. 1974, Dietrich et al. 1975). Periodontal ligament cells also produce PGE₂ even when unstimulated. This secretion is enhanced by IL-1 β , TNF- α and parathyroid hormone (Richards & Rutherford 1988, Saito et al. 1990a,b). LO and COX products (LTB₄, Thromboxanes and PGE₂, respectively) play important roles in systemic inflammation, endothelial cell activation and vascular endothelial growth factor (VEGF) expression and platelet aggregation.

Natural regulation of innate inflammation

Periodontal inflammation begins as a protective response to bacterial biofilm. In susceptible individuals, periodontal inflammation fails to resolve and chronic inflammation becomes periodontal pathology with systemic impact. The acute inflammatory response is protective, but a failure to remove inflammatory cells, especially neutrophils, characterizes the chronic, pathological lesion. The rapid and complete elimination of leucocytes from a lesion is the ideal outcome following an inflammatory event (Van Dyke 2007). Accordingly, inadequate resolution and failure to return tissue to homeostasis results in neutrophil-mediated destruction and chronic inflammation (Van Dyke & Serhan 2003), with destruction of extracellular matrix, and bone, scarring and fibrosis (Van Dyke 2008).

Efforts to control inflammation to date have been focused on the use of pharmacological agents that inhibit pro-inflammatory mediator pathways, for example, non-steroidal anti-inflammatory drugs (NSAIDs) (Serhan et al. 2007). NSAIDs target COX-1 and COX-2-dependent pathways inhibiting generation of prostanooids. Newer classes of inhibitors target lipoxigenase pathways and leucotriene (LT) production or TNF α .

The side-effect profiles of these agents prohibit their extended use in periodontal therapy and have been shown to have negative impact on the progression of systemic inflammatory conditions including CVD, diabetes and rheumatoid arthritis (RA).

More recent discoveries have uncovered eicosanoid pathways that signal the physiological end of the acute inflammatory phase (Levy et al. 2001, Van Dyke 2007). The eicosanoid product, lipoxins, are receptor agonists that stimulate the resolution of inflammation and promote the restoration of tissue homeostasis by limiting PMN migration into sites of inflammation, modulating the phenotype of macrophages, stimulating the uptake of apoptotic PMN without secretion of pro-inflammatory cytokines (Serhan et al. 1993, Maddox & Serhan 1996, Maddox et al. 1997).

Lipoxins are the natural pro-resolving molecules derived from endogenous fatty acids. Dietary fatty acids of the omega-3 class are also metabolized by similar pathways and the products (resolvins) have similar biological activity to lipoxins (Van Dyke 2007, Serhan & Chiang 2008). Resolvins stimulate the resolution of inflammation through multiple mechanisms, including preventing neutrophil penetration, phagocytosis of apoptotic neutrophils to clear the lesion, and enhancing clearance of inflammation within the lesion to promote tissue regeneration (Bannenberget al. 2005, Hasturk et al. 2007, Schwab et al. 2007).

In the context of inflammation, several animal studies with agonists of resolution of inflammation emphasize the importance of inflammation in the pathogenesis of systemic diseases. In type 2 diabetes models in mice, resolvins have been demonstrated to reverse insulin resistance and prevent complications of diabetes (Claria et al., 1998; Spite et al., 2009). Lipoxin levels in circulation have been directly linked to susceptibility to periodontitis and CVD in rabbits (Jain et al. 2003, Serhan et al. 2003) providing a potential link between the pathogenesis of these diseases.

Adaptive immunity

If the acute periodontal lesion persists without resolution, bacterial

antigens are processed and presented to the adaptive immune system by macrophages, and dendritic cells. Broadly, two subsets of lymphocytes recognize immune cell presented antigens of extracellular and intracellular pathogens; T lymphocytes and B lymphocytes. B lymphocytes bear immunoglobulin (Ig) molecules on their surface, which function as antigen receptors. Antibody (Ab), which is a soluble form of immunoglobulin, is secreted following activation of B cells to bind pathogens and foreign material in the extracellular spaces (humoral immunity). T cells are the effectors of cell-mediated immunity (delayed hypersensitivity). The T-cell antigen receptor is a membrane-bound molecule similar to immunoglobulin that recognizes peptide fragments of pathogens. Activation of the T-cell receptor requires the major histocompatibility complex (MHC), which is also a member of the immunoglobulin superfamily. Two classes of MHC molecules are required for activation distinct subsets of T cells. Various T-cell subsets kill infected target cells, and activate macrophages, B cells and other T cells.

Classically, T lymphocytes have been classified into subsets based on the cell surface expression of CD4 or CD8 molecules. CD4+ T-cells (T-helper cells) were initially subdivided into two subsets, designated Th1 and Th2, on the basis of their pattern of cytokine production. Th1 cells secrete interleukin-2 and interferon- γ (IFN- γ), whereas Th2 cells produce IL-5, IL-6, IL-4, IL-10 and IL-13. Both cell types produce IL-3, TNF α and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Kelso 1995, Zadeh et al. 1999). The major role of the Th1 cytokines IL-2 and IFN- γ is to enhance cell-mediated responses, whereas the Th2 signature cytokine IL-4 suppresses cell-mediated responses (Modlin & Nutman 1993). T-cell subsets are also important in the behaviour of B cells. For example, Th1 cells direct B cell secretion of immunoglobulin G2 (IgG2), whereas Th2 cells up-regulate IgG1 secretion. CD8 T cells (cytotoxic T-cells) are immune effector cells that also secrete cytokines that are characteristic of either Th1 or Th2 cells (Zadeh et al. 1999).

Two other well-defined CD4 T-cell subsets, Th17 and T-regulatory (Tregs) T-cells, play antagonistic roles as effector and suppressor cells respectively (Appay et al. 2008, Sallusto & Lanzavecchia 2009, Weaver & Hatton 2009). Th17 are named for their unique IL-17 production. Th17 cells also produce IL-22. Th17 lymphocytes, like Th1 cells, are also noted for their stimulatory role in osteoclastogenesis (Yago et al. 2009). Th17 cells are observed in chronic periodontitis sites, and Th17 related cytokines are produced in periodontal lesions (Takahashi et al. 2005, Vernal et al. 2005, Ohshima et al. 2009).

Tregs have a protective role in periodontal tissue damage. Natural Tregs are CD4 and CD25 expressing T cells that specifically regulate the activation, proliferation, and effector function of activated conventional T-cells (Appay et al. 2008, Belkaid & Tarbell 2009, Sallusto & Lanzavecchia 2009). Tregs are found in the periodontal disease sites (Nakajima et al. 2005, Cardoso et al. 2008). The cytokines produced by Tregs are TGF- β and T-lymphocyte-associated molecule 4 (CTLA-4), which down-regulate inflammation. IL-10, TGF- β and CTLA-4 are reported to decrease periodontal disease progression (Cardoso et al. 2008).

Macrophages, phagocytic cells from the myeloid lineage, efficiently ingest particulate antigen and express MHC class II molecules inducing T cells. Macrophages are widely distributed cells that play an indispensable role in homeostasis and defence. Dendritic cells also express MHC class II molecules and have co-stimulatory activity. It is evident that innate and adaptive systems are co-ordinately involved in the inflammatory response and tissue destruction, although we lack a complete understanding of the mechanism in many inflammatory conditions, including periodontitis, obesity, diabetes, RA and CVD.

The link to systemic conditions

Severe periodontal disease affects 10–15% of the general population and has been linked to cardiovascular disease in cross-sectional and cohort studies (Janket et al. 2003, Khader et al. 2003, Pussinen et al. 2005).

Studies reported that elevated cell and cytokine-mediated markers of inflammation, including C-reactive protein (CRP), fibrinogen and various cytokines are associated with periodontal disease (Black 2004). The same pro-inflammatory markers in periodontal disease have also been linked with atherothrombogenesis (Danesh et al. 2000). By reducing the progression of periodontal disease, levels of inflammatory markers common to both diseases (i.e. IL-6, TNF α and CRP) are decreased, which might in turn decrease vascular disease (Mustapha et al. 2007). It is still unknown whether inhibiting/reducing inflammation in general or CRP in particular will decrease the rate of vascular effects.

In several atherosclerosis studies using animal models, periodontal disease was shown to be a contributing factor (Jain et al. 2003, Gibson et al. 2004). Activated immune cells in the atherogenic plaque produce inflammatory cytokines (interferon, interleukin-1 and TNF α), which induce the production of substantial amounts of IL-6. These cytokines are also produced in various tissues in response to infection and in the adipose tissue of patients with the metabolic syndrome (Hansson 2005). IL-6, in turn, stimulates the production of large amounts of acute-phase reactants, including CRP, serum amyloid A, and fibrinogen, especially in the liver. Although cytokines at all steps have important biological effects, their amplification at each step of the cascade makes the measurement of downstream mediators such as CRP particularly useful for clinical diagnosis (Hansson 2005). Increased hsCRP plasma levels in patients with pre-hypertension and patients with established hypertension (Sesso et al. 2003) may link these two conditions. Major depression, physical inactivity, family histories of CVD and periodontal disease, advancing age and male gender are other risk factors for atherosclerotic CVD that are commonly found in patients with periodontitis and also may serve as confounders (Friedewald et al. 2009a,b, Genco & Van Dyke 2010).

Systemic inflammation, defined by increased circulating TNF- α , is associated with obesity and periodontitis and has been proposed as

a mechanism for the connection between these conditions (Al-Zahrani et al. 2003, Genco et al. 2005). A case-controlled study demonstrated that periodontitis is associated with elevated plasma triglycerides and total cholesterol (Loesche et al. 2005).

Summary and Recommendations

The critical and systematic reviews that have been performed to date, and those that will follow in this publication, suggest that periodontal disease is an independent predictor of several systemic conditions, including CVD, diabetes, PT-LBW infants, RA and cancer. Epidemiological studies, most retrospective, demonstrate the association. Animal studies of potential mechanism suggest biological plausibility; a very complex array of biological processes. Clinical proof of causality is elusive. Nonetheless, it remains clear from the data, that the three aspects of the pathogenesis of periodontal disease; infection, inflammation and adaptive immunity, all have a potential role and impact on the systemic inflammatory/immune response that either initiates or mediates a wide range of systemic diseases.

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