Inflammatory mechanisms linking periodontal diseases to cardiovascular diseases

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Schenkein HA, Loos BG. *Inflammatory mechanisms linking periodontal diseases to cardiovascular diseases.*

Abstract

Aims: In this article, inflammatory mechanisms that link periodontal diseases to cardiovascular diseases are reviewed.

Methods: This article is a literature review.

Results: Studies in the literature implicate a number of possible mechanisms that could be responsible for increased inflammatory responses in atheromatous lesions due to periodontal infections. These include increased systemic levels of inflammatory mediators stimulated by bacteria and their products at sites distant from the oral cavity, elevated thrombotic and hemostatic markers that promote a prothrombotic state and inflammation, cross-reactive systemic antibodies that promote inflammation and interact with the atheroma, promotion of dyslipidemia with consequent increases in pro-inflammatory lipid classes and subclasses, and common genetic susceptibility factors present in both disease leading to increased inflammatory responses.

Conclusions: Such mechanisms may be thought to act in concert to increase systemic inflammation in periodontal disease and to promote or exacerbate atherogenesis. However, proof that the increase in systemic inflammation attributable to periodontitis impacts inflammatory responses during atheroma development, thrombotic events or myocardial infarction or stroke is lacking.

Atherosclerosis and Periodontitis as Inflammatory Diseases

### Fundamentals of inflammation in atherogenesis and atherosclerosis (Fig. 1)

It is now widely accepted that a major component of pathology in cardiovascular disease (CVD) and particularly in atherosclerosis involves multiple components of the innate and adaptive immune systems leading to an inflammatory response within the atheromatous lesion (Libby et al. 2009). Links between periodontitis and atherosclerosis would be predicted based on inflammatory mechanisms initiated by bacteria associated with periodontal lesions, locally or systemically, that then influence the initiation or propagation of the atherosclerotic lesion. Such lesions may be initiated by inflammatory stimuli including systemic and locally produced inflammatory cytokines and chemotactic agents that cause changes in the endothelium such as up-regulation of adhesion molecules. These changes promote interactions with leucocytes, such as monocytes, that promote leucocyte migration into the intimal layer of the artery. Lipid streaks, comprised of modified low-density lipoproteins (LDL) within macrophages and dendritic cells (DCs) in the intimal layer, can initiate and propagate this inflammatory response. Up-regulation of the endothelium additionally leads to release of chemotactic cytokines such as monocyte chemotactic protein-1 (MCP-1) that further attract monocytes or other cells that can transport
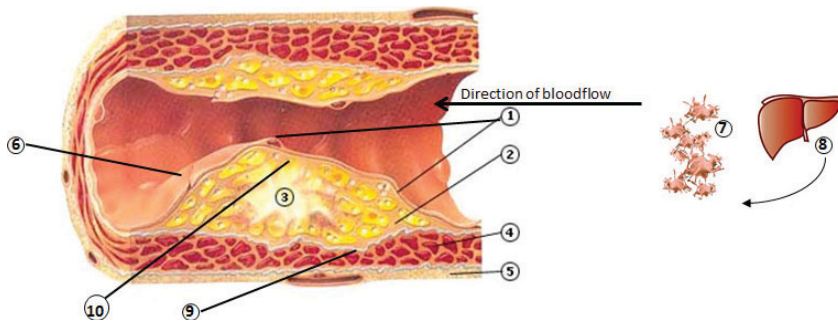
Fig. 1. 1. Dysfunctional endothelial lining: partial loss of integrity. ↑ adhesion molecules (ICAM-1, VCAM-1, E-selectin, P-selectin) and chemoattractants (e.g. IL-8, thrombin), increased platelet and leukocyte adhesion; diapedesis of monocytes, dendritic cells (both possibly with ingested bacteria) and T cells into the underlying inflammatory lesion. Activated platelets may form mini-thrombi. 2. Inflammatory lesion which may be in part initiated and/or propagated by bacteria originating from the periodontitis lesion, and also propagated by pro-inflammatory mediators (IL-1, IL-6, CRP, TNFα) and chemotactic factors (e.g. monocyte chemotactic protein-1) spilled over from the periodontal lesion, produced both in the liver and systemically. 3. Atheroma maturation. Lipid streaks and calcifications, comprised of modified low-density lipoproteins phagocytosed within macrophages/foam cells, resulting in ↑ pro-inflammatory cytokines (IL-6, IL-1, TNFa), ↑ chemoattractants (IL-8), ↑ matrix metalloproteinases (MMPs); the upregulated inflammatory lesion induces dysfunctional endothelial lining. CD4+ Th cells (↑IL-12,IL-18, IFN-γ) are also within the atheroma. 4. Smooth muscle cells (SMCs) and fibroblast with progressive fibrosis and loss of demarcation between inflammatory lesion and SMCs; development of a compensatory blood supply and increased outer muscle layer. 2 + 3 + 4 form the initimal layer of the artery. 5. Outer muscle layer. 6. Disintegrated endothelial lining (i.e. plaque rupture, exposition of underlying atherosclerotic plaque) generates thrombin from prothrombin, which in turn enzymatically generates fibrin from fibrinogen, →clotting cascade resulting in thrombosis, consequently to stroke or myocardial infarction. This process may be mediated by repeated bacteremias and pro-inflammatory state and activated inflammatory cells, such as from chronic periodontitis. 7. Activated platelets by bacteria in circulation from periodontal lesions, forming aggregates, the initiation of micro thrombus formation (out of scale with the whole picture). 8. ↑production of clotting factors in the liver due to inflammatory signals (IL-6), →pro-thrombotic state. Also increased production of acute phase reactants, including CRP, which will increase the pro-inflammatory state (out of scale with the whole picture). 9. Decreased collagen production and activation of MMPs resulting in reduction of SMC content and increased degradation of collagen that borders the fibrous cap, weakening the strength of the vessel, leading to fissuring of the atheroma. 10. In a progressed stage, the atheroma comprises of a large necrotic core which is exposed to the vasculature within the lesion, leading to contact with platelets, initiation of coagulation and ultimately, plaque rupture in so-called vulnerable lesions.

bacteria into the lesion. Thus, resident DCs in susceptible locations in the vasculature (Cybulsky & Jongstra-Bilen 2010), and monocytes attracted by chemotactic cytokines, become foam cells following ingestion of modified LDL. These cells release inflammatory cytokines, chemoattractants and matrix metalloproteinases (MMPs) which further enhance the inflammatory response in the lesion. CD4+ T cells are components of this lesion, comprised of predominantly Th1 cells which amplify the inflammatory process by producing, among other mediators, INF-γ (Libby et al. 2009). Thus, initiation and propagation of

early atherosclerotic lesions would theoretically be enhanced in periodontitis patients if periodontal microorganisms, or their effects on the host response such as initiating or propagating T-cell responses, contributed to endothelial dysfunction, modification in LDL, attraction and maturation of monocytes, enhanced uptake of lipids or attraction and promotion of Th1 T cell subsets (Andersson et al. 2010).

Maturation of the atherosclerotic lesion entails migration of smooth muscle cells (SMCs) into the intima, with progressive fibrosis. MMPs and other proteases promote SMC migra-

tion by degradation of the extracellular matrix. This permits proliferation of SMC and deposition of collagen and other proteins in the intimal layer. Thus, the mature atheroma is characterized by fibrosis and calcification (Raines & Ferri 2005). In addition, fibrosis in the atheroma may be enhanced by endothelial-mesenchymal transition stimulated by TGF-β, the production of which is promoted by inflammation (Kumarswamy et al. 2012). Such maturing lesions express increased levels of Th1 cytokines, including interleukin (IL)-12 and IL-18, which together further promote INF-γ production and additional inflammation as well as further up-regulation and dysfunction of endothelial cells, chemokine production (e.g. IL-8), cytokine production (e.g. IL-6) and MMP release.

Subsequent maturation of the atheroma, with development of a compensatory blood supply within the lesion, leads to additional activation and proliferation of inflammatory cells with inflammatory mediator release and generation of thrombin. Injury to the vasculature generates thrombin from prothrombin, which in turn enzymatically generates fibrin from fibrinogen, setting in motion the clotting cascade. Thrombin also interacts with receptors on a wide variety of cells to create a pro-inflammatory environment, with enhancement of SMC and fibroblast proliferation, platelet activation, interactions with cells of the innate and adaptive immune systems including monocytes, DCs, and lymphocytes, generation of mediators from endothelial cells, up-regulation of endothelial cell adhesion molecules such as ICAM-1, VCAM-1, E-selectin and P-selectin, and is chemotactic for monocytes and vascular SMCs. Thrombin generation is also associated with plaque rupture (Libby et al. 2009).

Plaque rupture, potentially leading to stroke or myocardial infarction, appears to be mediated by inflammatory cells (Imanishi & Akasaka 2012). Decreased collagen production and reduction of SMC content of the atheromatous lesion and increased degradation of collagen that comprises the fibrous cap by MMPs weaken the lesion, leading to fissuring of the atheroma. At this stage, the atheroma comprises a large necrotic core which is exposed to the vasculature within the lesion, leading

to contact with platelets, initiation of coagulation and ultimately, plaque rupture in so-called vulnerable lesions. Thus, the more advanced stages of atherogenesis could be impacted by periodontal inflammation and infection via effects on SMC migration, promotion of Th1 responses, thrombin generation and effects on collagen production and degradation. These processes could then promote rupture of the lesions and thrombus formation.

Rationale for mechanistic links

Epidemiological links between periodontitis and CVD indicate that there is an association between these two conditions (Lockhart et al. 2012). The proposed mechanistic links are summarized in Fig. 2. In Fig. 2, we propose that the host response to bacteremias may differ between patients with periodontitis due to individual variation in inflammatory pathways. It is conceivable that inherited genetic variation could enhance these potential mechanisms explaining the links between periodontitis and CVD.

Since both periodontitis and CVD are known to be inflammatory conditions, it has been proposed that inflammation due to periodontal microorganisms, the known aetiological agents of periodontitis, accounts for the contribution of periodontitis to increased CVD risk and severity.

We and others propose that the link between inflammation due to periodontal microbial pathogens and inflammatory responses that impact CVD may be manifested in several ways.

(1) A number of inflammatory mediators and markers are present in higher concentrations in the systemic circulation of patients with periodontitis than in periodontal healthy individuals. There are hypothetically two pathways by which this could occur:

(a) There are ample data indicating that inflammatory cytokines and other mediators are produced in the periodontal lesion (Preshaw & Taylor 2011). It has been hypothesized that these mediators could "spill over" into the circulation. If this does occur, and the mediators achieve sufficient concentrations with preservation of bioactivity, they would then impact tissues and organs distant from the oral cavity. In particular, these mediators from the periodontium could affect other organs, such as the liver, to initiate an acute-phase response that would impact other organs. This would lead to inflammatory changes in the endothelium such as up-regulation of adhesion molecules and promotion of cytokine production, and thus initiation or acceleration of atheroma development. It should be noted that there is not strong evidence supporting this mechanism for inflammatory cytokines and other mediators accessing the circulation (Teles & Wang 2011).

(b) It is well known that periodontitis patients have frequent bacteremic episodes and that detectable concentrations of lipopolysaccharide (LPS) are frequently found in the circulation. In addition, animal models of infection utilizing periodontal disease pathogens such as *Porphyromonas gingivalis* indicate that oral or systemic infection can promote inflammatory responses in sites distant from the oral cavity, such as in the atheroma
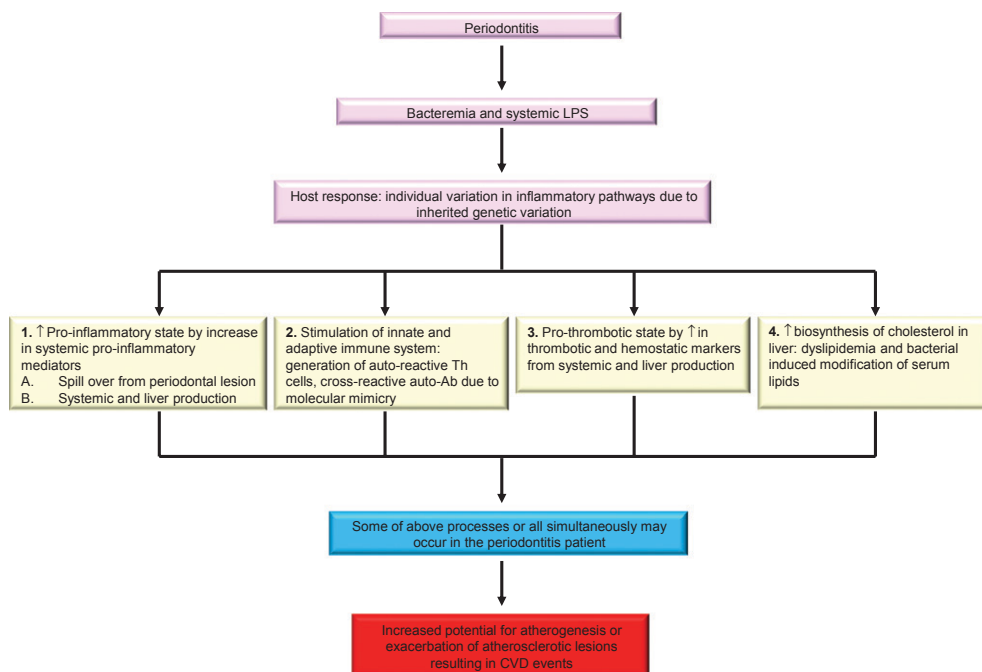

Fig. 2. Schematic overview of potential inflammatory mechanisms linking periodontitis to cardiovascular diseases.

(Gibson et al. 2006, Gibson & Genco 2007, Hayashi et al. 2010). Thus, bacteria, or their pro-inflammatory components, may stimulate systemic inflammatory responses as well as local inflammatory responses in atheromatous lesions (Teles & Wang 2011). This would follow their association with or modification of serum lipids, engagement of receptors on inflammatory cells and endothelium, invasion of endothelial cells, or seeding of atheromatous lesions with bacteria or bacterial components. Bacteria or their products could then promote inflammatory changes that would contribute to the development of atheromatous lesions.

(2) Several antibodies that may impact pathogenic inflammatory responses in atherosclerosis have been identified. Several of these antibodies are examples of "molecular mimicry" wherein cross-reactive antibodies induced by periodontal pathogens recognize host antigens and modulate their function. In some cases, these antibodies increase the risk for or accelerate atherosclerosis by enhancing endothelial inflammation, promoting uptake of lipids into macrophages, or blocking anti-atherogenic effects of protective molecules.

(3) Several studies indicate that serum concentrations of potentially inflammatory lipids, including LDLs, triglycerides (TGs) and very low-density lipoproteins (vLDLs) are elevated in periodontitis patients. These lipid subforms may more easily enter the blood vessel wall, may be more susceptible to modification and therefore more likely to be incorporated in to the atherosclerotic lesion. This would accelerate development of the local lesions and promote the maturation of the lesions.

(4) Some or all of these mechanisms together may be operant in individual patients with their summative effects impacting on cardiovascular inflammation.

A summary of these hypothesized mechanisms is presented in Fig. 2, emphasizing that some or all of them may be ongoing within periodontitis patients at any given time. What follows below in are summaries of studies that lend credence to these potential mechanisms, with emphasis on clinical studies that support, refute or illustrate the potential for these mechanisms to occur. We discuss the following:

(a) systemic biomarkers and inflammatory mediators noted to have particular relevance to the pathology of atherosclerosis,
(b) relevant thrombotic and hemostatic markers with known links to inflammatory processes,
(c) antibodies of relevance to atherogenesis that can be induced by oral microorganisms, and promote inflammation in the vasculature and the atheroma,
(d) serum lipids whose levels and potential modification by oral infection may influence atherogenesis and
(e) genetic markers that may explain individual variation in the inflammatory response in both periodontal infection and atherosclerosis.

Increased Systemic Mediators of Inflammation

A large number of studies demonstrate that there are increased circulating levels of inflammatory mediators in patients with periodontal diseases compared with healthy controls. Elevated levels of many of these mediators are statistically associated with increased cardiovascular risk and are therefore thought to be potential mechanistic links between periodontal infection and CVD, either as disease markers or as participants in inflammatory responses in endothelial tissue and atheromatous lesions. A summary of the studies discussed elsewhere in the article can be found in Table 1.

C-reactive protein

Of particular interest, and worth extra comment, is CRP. CRP is an acute-phase reactant that is mainly produced in the liver in response to a variety of inflammatory cytokines

such as IL-6. It therefore serves as a marker for systemic inflammation in a variety of conditions (Abd et al. 2011). Pertinent to periodontal diseases and their putative impact on CVD, serum CRP concentration has been proposed to be a risk marker for CVD and its serum levels are elevated in patients with periodontitis. However, the validity of serum CRP measurements as a risk predictor for atherosclerosis, and even its pathologic role in the development or progression of disease, is controversial (Anand & Yusuf 2010).

C-reactive protein, originally discovered due to its ability to bind to phosphorylcholine on pneumococcal C-polysaccharide, can bind to modified LDL, vLDL and the lipid mediator platelet-activating factor (PAF). CRP activates the complement system and is present in atheromas. Thus, it possesses properties implicating it as playing a direct role in the inflammatory responses attendant to atheroma formation. However, proof of a definitive role for CRP in the pathogenesis of atherosclerosis appears to be lacking (Ridker 2009).

The interpretation of data suggesting that elevated CRP levels in periodontitis is a link between periodontal inflammation and atherosclerosis depends on acceptance of the concept that CRP itself is pathological or that it influences downstream pathology impacting the atherosclerotic lesion. Alternatively, it may merely be a marker for systemic inflammation whose magnitude is modestly impacted by periodontal inflammation. Analysis of this controversial area is beyond the scope of this review, but arguments and evidence on either side of this issue are summarized in recent companion review articles (Anand & Yusuf 2010, Bisoendial et al. 2010)

CRP, IL-6 and other acute-phase reactants and inflammatory mediators in periodontitis

Levels of inflammatory mediators in periodontitis. There is ample evidence that serum CRP and other acute-phase reactant and inflammatory cytokine concentrations are higher in otherwise healthy individuals with chronic and aggressive periodontitis (AgP) than in periodontally healthy controls. Acute-phase reactants such

Table 1. Clinical studies suggesting the role of biomarkers and increased systemic mediators of inflammation in periodontitis as a link to inflammation in cardiovascular diseases (CVD)

Inflammatory mediator or marker	Association(s) with CVD	References
C-reactive protein (CRP)	Serum levels increased in chronic periodontitis (CP)	Ebersole et al. 1997, Loos et al. 2000, Slade et al. 2000, Noack et al. 2001, Offenbacher 2002, Ebersole et al. 2002, Buhlin et al. 2003, Beck and Slade et al. 2003, Amar et al. 2003, Yoshii et al. 2009, Gomes-Filho et al. 2011, Pejcic et al. 2011,
	Serum levels increased in aggressive periodontitis	Salzberg et al. 2006, Sun et al. 2009,
	Serum levels increased in patients with CVD and CP compared to either condition alone	Glurich et al. 2002, Persson et al. 2005, Amabile et al. 2008, Malali et al. 2010, Liu et al. 2010,
	Serum levels decrease along with improvement in surrogate measures of cardiovascular health [brachial artery flow-mediated dilation (FMD), hypertension, Framingham Risk Score] following therapy	Seinost et al. 2005, D'Aiuto et al. 2006, Higashi et al. 2008, 2009,
CRP, fibrinogen, interleukin (IL)-6, and other markers	Serum levels decreased following therapy	Iwamoto et al. 2003, D'Aiuto et al. 2004, Montebugnoli et al. 2005, Taylor et al. 2006, Hussain Bokhari et al. 2009, Vidal et al. 2009, Nakajima et al. 2010,
	Transient increase in serum levels shortly after therapy (24 h) and impairment of FMD	Tonetti et al. 2007,
	Serum levels unchanged following therapy	Ide et al. 2003, Yamazaki et al. 2005,
IL-6, haptoglobin, fibrinogen	Serum levels increased in CP	Buhlin et al. 2009,
IL-18, IL-4	Serum levels decreased in CP	
Serum amyloid A, alpha 1 anti-chymotrypsin	Serum levels increased in patients with CVD and CP compared to periodontitis alone	Glurich et al. 2002,
Matrix metalloproteinase-9	Serum levels decreased following therapy; high levels associated with changes in carotid IMT in CP	Behle et al. 2009, Soder et al. 2009,
Platelet-activating factor (PAF) and PAF-acetylhydrolase (AH)	PAF levels elevated in serum and gingiva, PAF-AH levels decrease following therapy	Noguchi et al. 1989, Losche et al. 2005, Zheng et al. 2006, Chen et al. 2010

as CRP and haptoglobin are elevated in chronic and AgP patients compared with healthy controls based on early studies (Ebersole et al. 1997, 2002), and later studies confirmed these findings (Amar et al. 2003, Yoshii et al. 2009). Further studies have provided evidence for elevated systemic levels of additional proinflammatory mediators and markers in chronic periodontitis (CP) including fibrinogen, haptoglobin, and IL-18, as well as decreased levels of anti-inflammatory mediators including IL-4 (Buhlin et al. 2009). Furthermore, CRP levels may be elevated in sera from patients with AgP based on two studies (Salzberg et al. 2006, Sun et al. 2009).

Since CVD and periodontitis share some common risk factors such as smoking, investigators have attempted to account for these covariates in further analyses of the these associations. Most studies observed that associations of serum levels of key mediators such as CRP and IL-6 with periodontitis remained significant following statistical correction for these factors (Loos et al. 2000, Noack et al. 2001, Buhlin et al. 2003, Gomes-Filho et al. 2011, Pejcic et al. 2011). Analysis of NHANES-3 data indicated that CRP is elevated in the US population in individuals with periodontal disease and in edentulous subjects after accounting for other explanatory variables for elevated CRP (Slade et al. 2000). Further, in a series of analyses of the Atherosclerosis Risk in Communities (ARIC) study, the association of CRP with periodontal measures such as pocket depth was observed following correction for a variety of CVD risk factors (Beck & Offenbacher 2002, Slade et al. 2003).

Levels of mediators in CVD patients with periodontitis. Since the observation that acute-phase reactants are elevated in periodontitis suggested a possible role for oral inflammation in the pathology of CVD, studies have been carried out that compared populations of patients with or without both disease entities. Most found that the levels of mediators such as CRP found in individuals with both CVD and periodontitis were additive relative to levels founds in patients with either condition (Glurich et al. 2002, Persson et al. 2005, Malali et al. 2010). For example, Glurich (Glurich et al. 2002), reporting data from the Erie County Periodontal Epidemiology Study, noted a hierarchy in levels of CRP, serum amyloid A and alpha 1-antichymotrypsin, wherein the highest levels were found in patients with both periodontitis and CVD compared to either condition alone. Other markers such as ceruloplasmin and sVCAM-1 were elevated only in CVD

while some including sICAM-1 were not elevated. Similar observations have since been made in other populations, including a Chinese population with relatively low CRP concentrations (Liu et al. 2010).

Impact of periodontal therapy on systemic inflammatory mediators. Periodontal therapy has been shown in several studies to decrease levels of some inflammatory and acute-phase markers, further implicating the periodontium as a source of systemic inflammatory mediators. Most such studies employed conservative therapies such as scaling, root planing and antibiotic treatment to show decreases in mediators such as CRP, TNF-α, and IL-6 (Iwamoto et al. 2003, D'Aiuto et al. 2004, Montebugnoli et al. 2005). In a study in which patients with advanced periodontitis were treated by full-mouth extraction, it was noted that there was a significant decrease in CRP as well as plasminogen-activator inhibitor-1, fibrinogen, and WBC counts within 12 weeks following treatment (Taylor et al. 2006). Subsequent treatment studies examining the impact of periodontal therapy on periodontitis patients with concurrent CVD noted similar reductions in key mediators of systemic inflammation (Hussain Bokhari et al. 2009, Vidal et al. 2009, Nakajima et al. 2010).

It is noteworthy that some studies failed to show changes in acute-phase reactants following periodontal therapy. Ide (Ide et al. 2003) observed that conservative periodontal therapy, including scaling and root planing, failed to alter serum levels of CRP, fibrinogen or inflammatory cytokines 6 weeks following treatment. In a study of Japanese subjects, it was observed that CRP and IL-6 levels at baseline were lower than had been previously reported in other populations, and that treatment did not significantly alter serum levels of these markers (Yamazaki et al. 2005). These studies may indicate that specific populations behave differently with respect to susceptibility to inflammatory stimulants or response to therapy, and that the relationships between periodontitis and CVD may not be uniform or universal.

Meta-analyses of CRP levels in periodontitis. A meta-analysis published in 2006 by Ioannidou (Ioannidou et al. 2006) failed to find a significant decrease in serum CRP levels due to scaling and root planing. A later systematic review which examined CRP levels in periodontitis and the impact of periodontal therapy on CRP levels was published in 2008 (Paraskevas et al. 2008). This analysis included studies utilizing the hsCRP assay in which the full range of serum CRP values can be evaluated, and which has been proposed to identify patients at risk for CVD (Ridker & Silvertown 2008). This review of cross-sectional case–control studies and of periodontal treatment studies indicated that there is ample evidence in the literature to conclude that CRP serum concentrations are elevated in individuals with periodontitis compared with control subjects, while there is a modest but statistically significant impact of therapy on CRP levels. Furthermore, in a comparison of standard treatment of scaling and root planing compared with intensive treatment in which antibiotic usage and an accelerated time-frame for therapy was included, there was no difference in result. Significantly, mean hsCRP levels in untreated patients with periodontitis exceeded the 3 mg/l threshold proposed as a cut-off for determination of elevated risk for CVD in otherwise healthy individuals.

Studies incorporating measures of cardiovascular outcome. Several studies examined outcomes related to cardiovascular health in tandem with measures of CRP and other inflammatory markers. In a study of the impact of periodontal therapy on endothelial dysfunction, as assessed by brachial artery flow-mediated dilation (FMD) (Seinost et al. 2005), improvement in FMD and a concomitant significant decrease in serum CRP levels were noted. In a trial comparing the impact of routine scaling and root planing with similar therapy that included local delivery antibiotics (D'Aiuto et al. 2006) it was observed that therapy that included antimicrobials resulted in enhanced reduction of CRP and IL-6 as well as enhanced clinical outcome in terms of decrease in blood pressure and Framingham Risk Score. Similarly, Highashi (Higashi et al. 2008, 2009) demonstrated impro-

ved endothelial function following periodontal therapy, with significantly decreased levels of CRP and IL-6, in patients with periodontitis. Interestingly, in a study that assessed serum inflammatory markers and FMD in patients following periodontal therapy, a significant increase in CRP, IL-6, sE-selectin and von Willebrand factor concentrations and impairment of FMD within 24 h after treatment was observed, noting that these effects were transient up-regulatory alterations of systemic inflammation (Tonetti et al. 2007).

Animal studies of inflammatory mediators. A limited number of animal studies have assessed inflammatory serum markers in models of CVD. In non-human primates, it has been demonstrated that induction and progression of ligature-induced periodontitis results in elevated acute-phase proteins CRP and fibrinogen, which was reversed following treatment (Ebersole et al. 2002). Administration of *aggregatibacter actinomycetemcomitans* or its LPS to ApoE$^{-/-}$ mice resulted in increased CRP as well increased small dense LDL and MMP-9 expression in the aorta (Tuomainen et al. 2008). Similarly, Zhang (Zhang et al. 2010) reported both elevation of the serum markers IL-6, IL-8, TNF-a, and MCP-1, as well as increased size of atherosclerotic plaques, in ApoE$^{-/-}$ mice infused with *A. actinomycetemcomitans*. A recent study sought to examine the impact of bacteremia with *P. gingivalis* on CVD by analysing mechanisms of inflammation within the myocardium (Akamatsu et al. 2011). It was observed that infusion of *P. gingivalis* into mice induced myocardial infarction or myocarditis. They further found that no inflammation was observed in mice genetically deficient in IL-17A suggesting a role for Th17 associated inflammatory pathways in *P. gingivalis*-induced cardiovascular inflammation.

Other markers: MMPs and PAF

Matrix metalloproteinases are thought to play a key role in both periodontal destruction (Page 1998) and in CVD due to their association with rupture of the atherosclerotic plaque, and can be induced by oral

bacterial products (Hajishengallis et al. 2002). It has been proposed that *P. gingivalis* proteases (gingipains) can both stimulate MMP production and process latent MMPs to become activated (Imamura et al. 2003). Thus, there is a hypothetical link between periodontitis and CVD through this pathway.

There are far fewer clinical studies implicating MMPs in the inflammatory link between periodontitis and CVD than for other mediators. Decreased serum MMP-9 levels in patients shortly following initiation of periodontal treatment has been noted (Behle et al. 2009), and associations between high MMP-9 and tissue inhibitor of metalloproteinase-1 (TIMP-1) concentrations in periodontitis patients and changes in carotid intima-media thickness (IMT) measures have been observed (Soder et al. 2009). Animal model studies of MMPs in this regard, which have examined both local and systemic associations between infection, MMPs, and atherogenesis, have given mixed results. For example, in a model of atherosclerosis induction by intravenous injection of *A. actinomycetemcomitans* in ApoE$^{-/-}$ mice (Tuomainen et al. 2008), it was observed that there was increased atherogenesis accompanied by increased expression of aortic MMP-9, increased serum gelatinase activity and decreased serum levels of pro-MMP-9 compared with un-infected control animals. However, it was observed in a model of *P. gingivalis*-induced bone loss following oral infection that particle size of HDL and vLDL is increased in MMP-8 deficient mice, implying that MMP-8 might play a protective, anti-inflammatory role with respect to systemic lipid profiles in *P. gingivalis* infection (Kuula et al. 2009). These limited data to date, utilizing differing modes of infection, fail to implicate MMPs as an important inflammatory factor linking periodontal infection and CVD.

Similarly, only a limited number of studies provide data supporting a hypothesis that PAF and PAF-acetyl hydrolase (PAF-AH), both associated with cardiovascular risk, are possible inflammatory factors linking periodontitis and CVD. PAF is known to be present at high levels in gingival tissue and gingival crevicular fluid (GCF) (Noguchi et al.

1989), and the PAF receptor is known to be a portal of entry for invasion of endothelial cells by certain bacteria including *A. actinomycetemcomitans* (Schenkein et al. 2000). It was observed (Losche et al. 2005) that serum concentrations of PAF-AH correlated with bleeding on probing, pocket depth and attachment level in periodontitis patients and that treatment reduced levels significantly. The authors suggested that these data, and those indicating that these mediators have been shown to be independent risk markers of CVD, indicate their importance in mediating periodontal effects on systemic disease. It has been further reported (Zheng et al. 2006) that sera from periodontitis patients contained significantly greater concentrations of PAF than samples from gingivitis or healthy control subjects. Furthermore, Chen (Chen et al. 2010) reported that serum PAF levels are elevated to the same extent in patients with periodontitis and with CVD.

Thus, although these inflammatory factors are known to be important in atherogenesis and outcomes such as stroke and myocardial infarction (MI), there are insufficient data at this point to implicate them in the link between periodontitis and CVD.

Thrombotic and Hemostatic Markers Influencing Inflammation

The coagulation and fibrinolytic systems are intimately associated with vascular inflammation and play an important role in atherogenesis and thrombosis (Davalos & Akassoglou 2012, Popovic et al. 2012). A number of hemostatic factors are associated with development of atherosclerosis including fibrinogen, von Willebrand factor, tissue plasminogen activator (tPA), plasminogen-activator inhibitor-1 (PAI-1), and factors VII and VIII.

Elevated fibrinogen is an indicator of systemic inflammation and is a risk marker for atherosclerosis, and results in increased blood viscosity and thus shear stress which can promote endothelial cell activation and platelet aggregation. Fibrinogen can interact with cellular integrin receptors CD11b/CD18 and CD11c/CD18 to stimulate production of proinflammatory cytokines or through TLR4

to induce MCP-1, MIP-1α and β, IL-6, IL-8, TNF-α, MMP-1 and MMP-9. Fibrinogen and its degradation products can be localized to atheromas as a structural component of the lesion where it, and its degradation products, can induce inflammatory cytokine production as well as promote platelet aggregation (Davalos & Akassoglou 2012).

The association of periodontitis with hemostatic factors has been reported by a number of investigators. An early report indicated that patients with periodontitis have higher plasma fibrinogen levels and white blood cell counts than age-matched controls, and suggested a link to myocardial infarction (Kweider et al. 1993). Subsequent studies likewise noted increased fibrinogen levels in periodontitis (Sahingur et al. 2003) including a report that there is an association between the number of periodontal pockets and fibrinogen levels, even after correction for a number of covariates associated with CVD risk and systemic inflammation (Schwahn et al. 2004). Subjects with >15 pockets had significantly elevated fibrinogen while, interestingly, edentulous patients did not demonstrate elevated levels. Full-mouth tooth extraction in patients with advanced periodontitis was reported to result in significant decreases in hemostatic factors including PAI-1 and fibrinogen (Taylor et al. 2006). In a cohort of patients with coronary artery disease (CAD), those with periodontitis had higher levels of fibrinogen as well as CRP and serum amyloid A than patients without CAD (Amabile et al. 2008). Elevated levels of fibrinogen were observed in patients with severe periodontitis (Buhlin et al. 2009), while decreased fibrinogen as well as IL-6 and CRP concentrations were found following periodontal therapy in patients with refractory hypertension (Vidal et al. 2009). Similarly, a decrease in plasma fibrinogen levels following non-surgical periodontal therapy in subjects with or without CVD has been observed (Hussain Bokhari et al. 2009). Alexander (Alexander et al. 2011) reported that gamma fibrinogen, an isoform of fibrinogen that may be associated with CVD, correlates with both CRP and the extent of gingival inflammation. Thus, clinical studies consis-

tently show that fibrinogen levels are elevated in periodontitis patients, even those with CVD, and are decreased following periodontal therapy.

Other thrombotic and hemostatic factors have also been implicated in the link between periodontitis and CVD. PAI-1 is a protease inhibitor that decreases fibrinolysis by inhibiting tPA and uPA (urokinase). These properties of PAI-1 are associated with increased risk for atherosclerosis. In a study that examined a variety of risk factors in periodontitis patients with CVD, significant but weak associations between periodontal indices and von Willebrand factor and PAI-1 levels were reported, but a follow-up study of the impact of scaling and root planing failed to note significant changes in levels of hemostatic factors (Montebugnoli 2005). It was speculated that this result may be due to the pre-existing CVD status of the subjects in this study and difficulty in modifying these factors in such patients. Bizzarro (Bizzarro et al. 2007) measured a series of thrombotic markers in periodontitis patients including PAI-1, vWF, prothrombin cleavage fragments, and D-dimer. They found that PAI-1 was elevated in patients with advanced periodontitis. Interestingly, a study by Bretz (Bretz et al. 2005) in an elderly population failed to demonstrate an association between PAI-1 levels and periodontitis despite significant increases in CRP, IL-6, and

TNF-a. The importance of these factors as links between periodontitis and CVD therefore remains an open question.

Platelets contribute to atheroma formation and thrombosis due to their aggregation, pro-inflammatory mediator release upon activation and their binding to thrombi at advanced stages of atheroma development and breakdown. Papapanagiotou (Papapanagiotou et al. 2009) examined platelet activation in periodontitis patients, by first measuring plasma concentrations of sP-selectin (sCD62P) and sCD40 ligand and then examining platelet-bound P-selectin and expression of activated glycoprotein IIb/IIIa. After adjustment for confounders, sP-selectin was significantly increased in periodontitis patients. Furthermore, the percentage of platelets expressing activated glycoprotein IIb/IIIa and the density of receptor expression, both indicating platelet activation, was elevated in periodontitis patients compared with controls and correlated with the proportion of teeth with >50% bone loss. Increased surface P-selectin on platelets from AgP patients as well as elevated CD18 on phagocytes resulting in increased aggregates of platelets with monocytes and polymorphonuclear leukocytes (PMNs) has also been reported (Fredman et al. 2011). These phenomena were reversed in the presence of Resolvin E1 implicating an impairment of inflammation resolution in these patients and increased

susceptibility to systemic inflammation (Fredman & Serhan 2011). Few corroborating animal studies relating thrombotic and hemostatic markers to periodontitis have been carried out. Ebersole reported an increase in plasma fibrinogen levels in experimental periodontitis in subhuman primates (Ebersole et al. 2002). Thus, there is some evidence for in vivo systemic platelet activation in periodontitis patients, but direct links to CVD risk due to periodontitis have not been studied.

A summary of clinical studies of thrombotic and hemostatic markers in periodontitis can be found in Table 2.

Antibodies

Patients with periodontitis are known to have elevated systemic antibody responses to a variety of periodontal microorganisms, and several such organisms are known to be able to induce cross-reactive and specific antibodies of relevance to atherosclerosis risk. These antibodies in turn may promote or influence inflammatory responses systemically and within atheromatous lesions. Measures of such antibodies have both been associated with increased cardiovascular risk in periodontitis.

Heat-shock proteins

Microbial heat-shock proteins (HSPs) and the immune response to

Table 2. Clinical studies suggesting a role of thrombotic and hemostatic mediators and markers in periodontitis as a link to inflammation in cardiovascular diseases (CVD)

Thrombotic or hemostatic marker or mediator	Association(s) with CVD	References
Plasminogen-activator inhibitor (PAI)-1	Serum levels in patients with advanced periodontitis decrease following full-mouth extraction	Taylor et al. 2006,
	Serum levels increased in chronic periodontitis (CP)	Bizzarro et al. 2007,
	No association of serum levels with periodontitis	Bretz et al. 2005,
Fibrinogen	Serum levels increased in CP	Kweider et al. 1993, Sahingur et al. 2003, Schwahn et al. 2004, Buhlin et al. 2009,
	Serum levels in patients with advanced periodontitis decrease following full-mouth extraction	Taylor et al. 2006,
	Serum levels decreased following periodontal therapy	Vidal et al. 2009,
	Serum levels increased in patients with CVD and CP compared to either condition alone	Amabile et al. 2008,
	Serum levels decreased following therapy in periodontitis patients with or without CVD	Hussain Bokhari et al. 2009,
von Willebrand factor and PAI-1	Significant association with periodontal measures in periodontitis patients with CVD	Montebugnoli 2005
sP-selectin, P-selectin, CD18, activated glycoprotein IIb/IIIa (platelet activation markers)	Plasma and cell-surface levels elevated in periodontitis, reversed by resolvin	Papapanagiotou et al. 2009, Fredman et al. 2011, Fredman & Serhan 2011

these proteins represent a hypothesized pathway linking bacterial infections and atherosclerosis. Human HSPs are molecular chaperones that transport protective proteins to the cell surface. Stressed human tissues, such as those at a site of inflammation, will express HSPs which in turn are subject to regulation by both innate and adaptive arms of the immune system. For example, HSP-reactive T-cells can be found in the circulation and in atherosclerotic lesions, and anti-HSP reactive antibodies can be detected in serum of patients with atherosclerosis. In addition, HSPs can interact directly with TLRs and thereby induce inflammatory responses in macrophages and endothelial cells.

Most bacteria also express stress-induced antigens that sufficiently resemble human HSPs so as to be able induce the production of antibodies and T-cells that react with human HSPs. This form of molecular mimicry, via induction of cross-reactive cells and antibodies, may be a link between infection and atherosclerosis. A scenario whereby periodontal microorganisms can induce inflammatory responses via induction of immunity may be the following. Following up-regulation of endothelial cell HSP60 due to the stress of well-known risk factors such as high blood cholesterol levels, modified LDL, hypertension, diabetes, and smoking, patients with pre-existing bacterial infections may have elevated levels of cross-reactive anti-HSP antibodies and circulating lymphocytes. Along with true autoantibodies and autoreactive T-cells, these can infiltrate early atherosclerotic lesions and enhance the inflammatory response. Oral pathogens express such antigens and are capable of inducing such responses to enhance inflammation in the atheroma (Van Eden et al. 2007).

Periodontal pathogens including *P. gingivalis* express HSPs such as HSP60 (GroEL) (Lu & McBride 1994, Maeda et al. 1994, Vayssier et al. 1994). In periodontitis patients, GroEL stimulates inflammatory cytokines from macrophages via TLR (Ueki et al. 2002) and anti-*P. gingivalis* GroEL is elevated compared to healthy individuals. Patients with mild periodontitis have elevated serum levels of HSP60 and elevated small dense LDL compared to healthy controls. Serum HSP60 concen-

trations correlate directly with serum TG levels and inversely with HDL levels (Rizzo et al. 2012). Elevated serum levels of anti-*Fusobacterium nucleatum* GroEL has also been described in periodontitis patients (Lee et al. 2012). *Fusobacterium nucleatum* GroEL itself has properties consistent with atheroma formation and pathology including enhanced foam cell formation, activation of endothelial cells with increased monocyte adhesion and migration and promotion of coagulation (Lee et al. 2012).

Cross-reactivity of bacteria-stimulated anti-HSPs with human HSPs would suggest that they could react with human HSP60 expressed on endothelial cells. Anti-*Tannerella forsythia*, anti-*A. actinomycetemcomitans*, and anti-*P. gingivalis* HSPs are cross-reactive with each other and with human HSP (Hinode et al. 1998). *Porphyromonas gingivalis* HSP60 contains both B- and T-cell epitopes cross-reactive with hHSP60 (Choi et al. 2004). Furthermore, T-cell lines derived from atherosclerotic plaques are cross-reactive between human HSP and GroEL (Ford et al. 2005). In addition, antibody levels to GroEL and human HSP60 were found to be higher in atherosclerosis patients in comparison to periodontitis patients and healthy subjects, and GroEL-specific T-cells were detected in both the circulation and in some atherosclerotic lesions in atherosclerosis patients (Yamazaki et al. 2004). In addition, T-cell lines established from atherosclerotic plaques that are specific for GroEL and for human HSP60 have similar cytokine profiles and phenotypic characteristics to *P. gingivalis*-specific lines from periodontitis patients (Ford et al. 2005). These studies support the notion that bacterial HSP can induce immune responses that would be expected to promote inflammation in the atheroma itself.

Finally, animal studies support a potential interaction of bacterial HSPs and promotion of atherosclerosis. Mori (Mori et al. 2000) found that immunization of mice on a high-cholesterol diet with human HSP increased both fatty streak formation and periodontal inflammation, and Lee et al. (2012) found that immunization of ApoE$^{-/-}$ mice with *F. nucleatum* GroEL promoted

increased lipid deposition in atherosclerotic plaques in the aorta. These studies thus support the ability of anti-HSP induced either by human or bacterial immunogens to interact with both periodontal and atheromatous tissues.

Anti-cardiolipin

Pathogenic anti-cardiolipin (anti-CL) antibodies, most commonly found in patients with systemic lupus erythematosis (SLE) or the anti-phospholipid syndrome (APS), are known to be associated with systemic sequelae that include vascular thrombosis and early atherosclerosis. The target antigen for these autoantibodies is on the serum protein β2-glycoprotein 1 (β2GP1), which binds to anionic lipids such as cardiolipin to form a complex that is recognized by these antibodies. It is thought that a physiological function of β2GP1 may be to protect damaged endothelial cell surfaces from promoting inappropriate coagulation, though the function of this protein is still not clearly established. It is believed that anti-CL disrupts this protective function. In vitro treatment of endothelial cells with anti-CL leads to up-regulation of adhesion molecules and production of inflammatory cytokines, thus potentially linking the presence of these antibodies to enhanced vascular inflammation. In addition, the binding specificity of β2GP1 is not limited to cardiolipin; β2GP1 also binds to modified LDL and can be detected in atheromatous lesions. Hence, anti-CL binding to β2GP1 may increase risk for early CVD in SLE patients. This phenomenon termed "autoimmune atherosclerosis" entails uptake of modified lipids into macrophages and promotion of inflammatory mechanisms resulting from the formation of these immune complexes within the atheroma (Kobayashi et al. 2005, Matsuura & Lopez 2008).

It has been shown that a variety of microbial pathogens are capable of inducing pathogenic anti-CL because of their similarities to peptide sequences in β2GP1. One such sequence in β2GP1 is TLRVYK, and many microorganisms contain homologous peptide sequences to TLRVYK. It has been hypothesized that some anti-CL found in patients without autoimmune disease

may result from molecular mimicry of microbial origin (Blank et al. 2002). In fact, Wang (Wang et al. 2008) demonstrated that patients with *A. actinomycetemcomitans* infection displayed elevated antibody concentrations to the peptide SIRVYK, a sequence found in *A. actinomycetemcomitans* that is homologous to TLRVYK. Furthermore, anti-SIRVYK correlated with anti-TLRVYK in patients colonized with *A. actinomycetemcomitans*. Thus, it was reasoned that infection with *A. actinomycetemcomitans* contributes to the content of antibody reactive with β2GP1.

Patients with chronic or AgP demonstrate a higher prevalence of elevated levels of anti-CL than healthy subjects without periodontitis (Schenkein et al. 2003). Between 15% and 20% of periodontitis patients contain elevated levels IgG or IgM antibodies, raising questions as to the source of these antibodies. In addition, Gunupati (Gunupati et al. 2011) has shown that periodontal therapy in patients who have experienced an acute myocardial infarction leads to significant decreases in serum concentrations of IgG and IgM anti-CL. These studies, and those demonstrating cross-reactivity between oral bacterial antigens and β2GP1, implicate the oral microflora as a source of anti-CL.

Additional clinical studies have shown an association between levels of serum anti-CL and serum markers of vascular inflammation, including sICAM-1, sVCAM-1, and sE-selectin, in periodontitis patients (Schenkein et al. 2007). Furthermore, Turkoglu (Turkoglu et al. 2008) demonstrated that periodontitis patients with hypertension have elevated IgM anti-CL serum antibodies and speculated that these antibodies may impart increased risk for atherosclerosis in these patients. Finally, Pussinen (Pussinen et al. 2004b) noted that in patients with severe periodontitis and high serum LPS concentrations, periodontal therapy resulted in a decrease in serum anti-β2GP1 concentration, implicating gram negative bacterial infection in the production of anti-β2GP1 in periodontitis.

Other antibodies

Other antibodies that link periodontitis with CVD include anti-phosphorylcholine (anti-PC) and anti-oxidized LDL (anti-oxLDL). These have the common attributes that they have been shown to be inducible by periodontal pathogens and both react with neoepitopes revealed on LDL following modification. Antibodies of both specificities have been implicated in both protection against and risk for CVD.

IgM anti-PC is a component of the innate immune system and thus present in all sera (Briles et al. 1987, Scott et al. 1987). In addition, IgG anti-PC is present at higher levels in sera from patients with periodontal attachment loss than in healthy subjects and is locally produced in the periodontal lesion (Schenkein et al. 1999). IgG anti-PC is cross-reactive between many oral bacteria and oxLDL (Schenkein et al. 2001, 2004). In mice, high levels of IgM anti-PC is associated with protection against atherosclerosis (Binder et al. 2003, Shaw et al. 2003), but this has not been demonstrated in humans. Thus, the overall impact of elevated levels of anti-PC due to stimulation with periodontal bacterial antigens is not known.

Anti-oxLDL is present in sera of patients with CVD and has been proposed to be a marker for cardiovascular risk. Such antibodies are also locally produced in the gingiva and present in GCF (Schenkein et al. 2004). It has been recently demonstrated that natural IgM antibodies and monoclonal antibodies raised against modified LDL recognized epitopes on the arginine-specific gingipain (Rgp) of *P. gingivalis*. These are not phosphorylcholine-containing epitopes but rather unique antigens that cross-react with modified LDL (Turunen et al. 2012).

It has been observed that the serum levels of anti-oxLDL were significantly higher in CVD patients with greater severity of periodontal disease (Montebugnoli et al. 2004, 2005), and Monteiro (Monteiro et al. 2009) demonstrated that periodontitis patients' sera contain higher concentrations of antibodies against oxidized LDL than healthy controls. It has been proposed that oxLDL that is opsonized by antibodies such as anti-PC, anti-oxLDL and anti-CL, could promote systemic inflammation through interactions with DC's, which produce IL-12 and IL-18 leading to IFN-γ production. The production of IL-12 in this manner, along with stimulation by oxLDL-containing immune complexes, could lead to monocyte maturation and promotion of inflammatory responses in the endothelium, as well as increased foam-cell formation. Since oral bacteria can induce these cross-reactive antibodies, and they in turn bind to these bacteria, this represents a potential mechanistic link between periodontal infection and inflammation in the atheroma (Tew et al. 2012).

In summary, cross-reactive and autoreactive antibodies can enhance or even precipitate chronic inflammatory reactions in early or advanced atherosclerotic lesions. A major source for the generation of these antibodies seems to be the microbiota of periodontal infections and mimicry of the host antigens in periodontitis. These studies are summarized in Table 3.

Dyslipidemia, Lipid Peroxidation and Inflammation

Increased serum concentrations of total cholesterol or especially subsets of serum lipids including LDL, vLDL, and TGs are considered pro-atherogenic. LDLs, which diffuse freely into the intimal layer of blood vessels, can be modified in a number of ways, including by oxidative or proteolytic mechanisms, so as to be recognizable by cellular receptors on phagocytes. Thus, macrophages in the subendothelial environment can become engorged with modified lipids to become foam cells during the early stages of atheroma formation. Activation of macrophages, with release of inflammatory mediators, can stimulate endothelial cells to release chemotactic cytokines such as MCP-1 and to up-regulate cell-surface receptors involved in further monocyte recruitment into the atheromatous lesions. Clinical studies have demonstrated dyslipidemia in periodontitis patients that may be reduced following periodontal therapy, implicating periodontal inflammation as a link to atherogenesis. Furthermore, a limited number of in vitro studies in animal models indicate that periodontitis is associated with changes in serum lipid concentrations and with lipid modification that would favour atherogenesis.

Table 3. Studies implicating antibodies in periodontitis as a link to inflammation in cardiovascular diseases (CVD)

Antibody	Association(s) with CVD	References
Anti-heat-shock proteins (HSPs)	Periodontal pathogens express HSPs such as HSP-60 (GroEL)	Lu & McBride 1994, Maeda et al. 1994, Vayssier et al. 1994,
	Anti-*Porphyromonas gingivalis* GroEL and anti-*Fusobacterium nucleatum* GroEL, elevated in periodontitis patients, stimulates inflammatory cytokine production as well as monocyte and endothelial cell activation	Ueki et al. 2002, Lee et al. 2012,
	Serum HSP60 correlates with serum triglycerides, inversely with HDL levels	Rizzo et al. 2012,
	Anti-*Tannerella forsythia*, anti-*Aggregatibacter actinomycetemcomitans* and anti-*P. gingivalis* HSPs are cross-reactive with each other and with human HSP	Hinode et al. 1998,
	T-cell lines derived from human atheromas specific for GroEL and HSP60 functionally similar to *P. gingivalis*-specific T-cell lines from periodontitis patients	Ford et al. 2005,
Anti-cardiolipin (anti-CL)	Elevated levels in periodontitis patients	Schenkein et al. 2003,
	Elevated levels in periodontitis patients with hypertension	Turkoglu et al. 2008,
	Serum levels decreased following periodontal therapy	Gunupati et al. 2011, Pussinen et al. 2004b,
	Elevated serum levels in periodontitis patients associated with elevated serum markers of endothelial cell activation	Schenkein et al. 2007,
	Periodontal pathogens can produce antibodies cross-reactive with anti-CL	Wang et al. 2008,
Anti-phosphorylcholine (PC)	Elevated levels in periodontitis patients, cross-reacts with oxidized low-density lipoproteins (oxLDL) and oral bacteria	Schenkein et al. 1999, 2001, 2004,
Anti-oxLDL	Locally produced in gingiva, found in GCF	Schenkein et al. 2004,
	Cross-react with *P. gingivalis* Rgp protease	Turunen et al. 2012,
	Higher in serum of periodontitis patients, and in periodontitis patients with CVD	Montebugnoli et al. 2004, 2005, Monteiro et al. 2009

Relationships between infection, serum lipid levels and structure and inflammatory mechanisms affecting atherogenesis suggest mechanisms linking periodontitis and CVD. Although cholesterol biosynthesis and transport are fundamental physiological processes, the appearance and properties of serum lipids are influenced by infectious processes. Pertinent to periodontitis, the presence of LPS in plasma and acute-phase responses to systemic dissemination of bacteria could promote elevated biosynthesis of cholesterol in the liver, which in turn is transported as serum lipids capable of binding to bacterial LPS. In this manner, a pathway can be envisioned in which periodontal infection both promotes dyslipidemia and interacts with serum lipids so as to enhance their atherogenicity.

Clinical studies of systemically healthy and diabetic patients demonstrated associations between periodontitis and dyslipidemia. Losche et al. (2000) reported that CP patients exhibited higher levels of total cholesterol and serum LDL than control subjects. This observation has been repeated in other studies (Katz et al. 2001). Nibali et al. (2007) observed elevation in serum LDL levels as well as decreases in HDL levels in periodontitis patients who were otherwise healthy, along with significantly elevated white cell counts. These relationships were seen in non-smokers as well as in smokers. Comparison of serum lipid levels between patients with CP and healthy controls revealed increased TGs and decreased HDL concentrations in CP, as well as increased serum levels of oxLDL and modified LDL (Monteiro et al. 2009). Studies evaluating the density of LDL in periodontitis patients have also found higher levels of atherogenic small dense LDL in CP patients (Rizzo et al. 2012) and in AgP patients (Rufail et al. 2007). In diabetic subjects, Nishimura (Nishimura et al. 2006) observed that total cholesterol and serum LDL was associated with antibody titre to *P. gingivalis*. Other investigators, however, have failed to observe these relationships (Machado et al. 2005, Valentaviciene et al. 2006).

Several studies have examined modification of serum lipid profiles via periodontal therapy. Losche et al. (2005) did not observe alterations in total cholesterol, LDL, HDL, or TGs following therapy, while Oz (Oz et al. 2007) and Duan (Duan et al. 2009) found that total cholesterol and LDL levels decreased. Montebugnoli observed decreased circulating oxLDL levels following intensive periodontal therapy (Montebugnoli et al. 2005).

Proposed mechanisms that link alteration of lipid profiles due to periodontal inflammation to enhanced inflammatory mechanisms in CVD suggest interactions with serum lipids that promote enhanced uptake of modified lipids by macrophages. A series of studies by Pussinen and co-workers have suggested pathways connecting periodontal and cardiovascular inflammation. They isolated serum lipids from periodontitis patients before and after periodontal therapy and determined their uptake in vitro by macrophages. They found that the concentration of oxLDL as well as the production of TNF-α by macrophages correlated with serum LPS concentrations. In addition, lower concentration of HDL, smaller particle size of LDL and induction of inflammatory cytokines by LDL were found in patients with more severe periodontitis (Pussinen et al. 2004b). Treatment effects were dependent on baseline LPS levels, with increases in HDL and LDL particle size following therapy. Using

similar methodologies, they found that HDL promoted the enhanced efflux of cholesterol from macrophages following therapy, especially in patients in which CRP levels were also reduced by periodontal treatment (Pussinen et al. 2004a). Kallio (Kallio et al. 2008) observed that in periodontitis patients, elevated serum LPS levels persisted following therapy and that LPS was associated with highly atherogenic lipids, including vLDL and intermediate density lipoproteins (IDL). Thus, LPS associates with proatherogenic lipids in periodontitis, and this pattern is not changed following treatment, indicating that the disease, or colonization by Gram negative bacteria, persisted even after therapy.

Further mechanisms whereby periodontitis could impact the atherogenicity and proinflammatory potential of serum lipids were suggested by in vitro studies. A series of studies by Kuramitsu (Kuramitsu et al. 2003, Miyakawa et al. 2004) demonstrated the ability of *P. gingivalis* to induce in vitro foam cell formation in the presence of exogenous LDL. This was replicated by other investigators and a role for *P. gingivalis* fimbriae (Giacona et al. 2004) and proteases (Miyakawa et al. 2004) was confirmed for these in vitro phenomena. Miyakawa demonstrated aggregation of LDL by *P. gingivalis* and outer membrane vesicles, degradation of ApoB-100, and enhanced foam cell formation (Miyakawa et al. 2004).

The ability of periodontal pathogens, such as *P. gingivalis*, to induce atheroma formation in animal models, has been established (Li et al. 2002, Jain et al. 2003). These models have been used to study the impact of periodontal infection on lipid levels and lipid modification leading to increased atherogenesis. In a study of the impact of intravenous administration of *P. gingivalis* into ApoE^{-/-} mice, enhanced atheroma production and increases in serum cholesterol and LDL and decreased HDL were noted (Hashimoto et al. 2006). It was further noted that incorporation of protease inhibitors, or an Rgp-defective mutant of *P. gingivalis*, led to decreased atheroma formation implicating *P. gingivalis* protease in the response. This study demonstrated the ability of Rgp of *P. gingivalis* to modify apoB-100 (the primary apolipoprotein component of LDL), which leads to increased uptake of LDL into macrophages. Likewise, Maekawa (Maekawa et al. 2011) observed increased atheroma formation in ApoE^{-/-} mice utilizing an oral infection model, noting elevation in LDL, vLDL and total cholesterol as well as decreased HDL. They noted that these changes do not occur in wild-type mice implying the importance of a susceptible host in this model. Uchiumi (Uchiumi et al. 2004) noted that chronic infusion of LPS in rats increased serum TG levels.

In summary (Table 4), there is evidence from clinical studies that patients with periodontitis can demonstrate elevated levels of serum cholesterol as well as of LDL, small dense LDL, vLDL, IDL, and TGs, in concert with decreased levels of HDL, thus presenting with a more atherogenic lipid risk profile. Studies show that patients with periodontitis have low levels of circulating LPS, as well as episodes of bacteremia, and that LPS can be circulating in a bound form to atherogenic serum lipids. In addition, oxLDL can be found at higher levels in periodontitis. Both oxLDL and LPS-LDL are modified forms of lipid that would tend to enhance lipid uptake into macrophages to enhance the inflammatory response in the atheroma. These concepts are supported by in vitro demonstration of modification of lipids due to association with LPS and proteolytic modification of lipids by bacterial proteases with enhanced formation of foam cells. Finally, animal models indicate that similar alterations in the lipid profile can occur due to infections with periodontal pathogens and that promotion of atheroma development can occur in animals prone to hyperlipidemia due to genetic factors or dietary factors.

Common Genetic Risk Factors Impacting Inflammation

Of interest are recent findings that suggest a common genetic background for CVD and periodontitis, which could dictate the way the host responds in general to certain types of inflammatory processes. The underly-

Table 4. Studies implicating the role of serum lipids in periodontitis patients as a link to inflammation in cardiovascular diseases (CVD)

Lipid	Association(s) with CVD	References
Total serum cholesterol, serum lipid profile	Chronic periodontitis (CP) patients have higher total serum cholesterol than controls	Losche et al. 2000, Katz et al. 2001, Nibali et al. 2007,
	Periodontal therapy can improve serum lipid profiles	Pussinen et al. 2004b, Montebugnoli et al. 2005, Oz et al. 2007, Duan et al. 2009,
Low-density lipoprotein (LDL)	CP patients have higher serum LDL than controls	Losche et al. 2000, Katz et al. 2001, Nibali et al. 2007,
	CP patients have higher oxidized LDL than controls	Monteiro et al. 2009,
	Chronic and aggressive periodontitis patients have higher small dense LDL than controls	Pussinen et al. 2004b, Rufail et al. 2007, Rizzo et al. 2012,
	Porphyromonas gingivalis induces in vitro foam cell formation in the presence of exogenous LDL	Kuramitsu et al. 2003, Miyakawa et al. 2004, Giacona et al. 2004, Miyakawa et al. 2004,
High-density lipoprotein (HDL)	CP patients have lower HDL than controls	Nibali et al. 2007, Monteiro et al. 2009, Pussinen et al. 2004b,
Triglycerides (TGs)	CP patients have higher serum TGs than controls	Monteiro et al. 2009,
Very (v)LDL, intermediate density lipoprotein (IDL)	In CP, serum LPS is associated with atherogenic lipids (vLDL, IDL), and remains so after periodontal therapy	Kallio et al. 2008

ing notion is that periodontitis and CVD are both associated with common inflammatory mechanisms or that they interact by influencing inflammatory processes that impact the disease process. Several susceptibility loci for CVD have been identified by various genome-wide association studies (GWAS) (Consortium, W.T.C.C 2007, Helgadottir et al. 2007, McPherson et al. 2007, Samani et al. 2007), and the *ANRIL* locus is the best replicated coronary heart disease associated risk locus to date (Schunkert et al. 2008, 2011, McPherson 2010, Palomaki et al. 2010). In addition, variants within this region were independently found to be associated with type 2 diabetes (Saxena et al. 2007, Scott et al. 2007, Zeggini et al. 2007), abdominal aortic and intra-cranial aneurysms (Helgadottir et al. 2008), ischaemic stroke (Matarin et al. 2008), Alzheimer's disease and vascular dementia (Emanuele et al. 2011) and high-grade glioma susceptibility (Wrensch et al. 2009). Significantly, Schaefer et al. (2011, 2009), and replicated by Ernst et al. (2010), observed a highly increased risk for AgP with variants of *ANRIL*.

ANRIL is a long intergenic non-coding RNA within the Chr9p21 locus. *ANRIL* is expressed in tissues and cell types that are affected by atherosclerosis. Long intergenic non-coding RNAs, like *ANRIL*, do not have an obvious open reading frame and are arbitrarily considered longer than 200 nucleotides (Mercer et al. 2009). These RNAs have diverse cellular functions and regulate gene expression by RNA–RNA, RNA–DNA, or RNA–protein interactions (Hung & Chang 2010). On the basis of functional studies, it is suggested that *ANRIL* might constitute a regulator of epigenetic modification and gene expression and thereby modulate cardiovascular risk (Holdt & Teupser 2012); its role in inflammatory processes is still elusive. Nevertheless, similar processes might play a role in periodontitis, thus yielding the possibility that these two diseases have common inflammatory pathways and in this way, a certain proportion of the population might be susceptible to both diseases. The causative variant(s) and functional role of ANRIL are as yet unknown despite extensive fine-mapping and functional studies (Schunkert et al.

2008, Jarinova et al. 2009), and therefore a causal role for *ANRIL* variants in both periodontitis patients and CVD patients is not yet determined; a role in inflammatory pathways seems logical. Another important difficulty we face to date is the limited evidence of increased frequencies of *ANRIL* variants in patients with CP, who often tend to be at ages when cardiovascular events take place. The frequency of some *ANRIL* variants was significantly higher in Dutch CP individuals, while not significantly increased in German CP patients (Schaefer et al. 2011). Also, it remains to be determined what *ANRIL* gene variant frequencies are in both AgP and CP populations (and respective controls) of other ethnic descents than Northern Europe. Further research will shed light on the functional significance of these as of yet limited, but promising observations and of the contribution of genetic risk to the relationship between periodontitis and CVD.

Concluding Remarks: Periodontal Inflammation, Systemic Inflammatory Mediators, Immune Mediators and CVD, Alternative Hypotheses

The preponderance of the data appears to support the concept that periodontitis can contribute to systemic levels of inflammatory mediators and markers associated with increased risk for CVD. Studies in this regard support the concept that a variety of mechanisms that depend on exposure of the oral microflora or components thereof to organs distant from the oral cavity are likely to account for these findings. Such organs likely include the liver, elements of the innate and adaptive immune systems, components of the coagulation and fibrinolytic systems, and the atheromatous lesion itself, leading to enhanced systemic levels of inflammatory mediators.

In otherwise healthy periodontitis patients, CRP levels are generally above the level shown in epidemiologic and intervention studies to be associated with elevated risk for CVD (Ridker & Silvertown 2008). Treatment studies appear to show (Paraskevas et al. 2008) a modest decrease in CRP, indicating that either periodontitis makes a modest

contribution to CRP levels in patients with other predisposing factors to systemic inflammation, or that end-points of periodontal therapy are difficult to reach even with aggressive treatment. The contribution of CRP mechanistically to the disease process of CVD is itself debatable, despite many biological functions of CRP that conceptually can be thought to be operant in disease. Thus, this mechanistic linkage remains open.

Numerous inflammatory reactions, with release of a myriad of mediators, occur in the periodontal tissues. It has been proposed that local periodontal lesions might produce sufficient quantities of relevant mediators that enter the circulation so as to enhance their levels, but incisive studies demonstrating that this occurs are not available. Alternatively, it is certainly the case that periodontitis patients have frequent bacteremias and that sera from such individuals contain elevated LPS. Thus, promotion of a systemic inflammatory response with production of CRP or other mediators most likely occurs.

What appears to be missing in the mechanistic argument that periodontal disease causes systemic inflammation leading to increased atherogenesis, or increased risk for a cardiovascular event, is proof that the increase in systemic inflammation attributable to periodontitis impacts the atheromatous lesion or occurrence of thrombosis. Studies of periodontal therapy, some of which demonstrate an impact on surrogate measures of CVD including systemic levels of inflammatory mediators, have not addressed CVD end-points that would be convincing in this regard. A recent systematic review (Lockhart et al. 2012) correctly noted that there are numerous papers demonstrating hypothetical links between CVD and periodontitis, mainly through studies showing correlations between these markers and both diseases. However, the absence of prospective clinical trials and incisive studies that show causal relationships and implicate specific pathways have not been carried out. In addition, the concept that elevation of key markers such as CRP has a mechanistic role in CVD inflammation, despite their

diagnostic or prognostic value, is not itself proven or universally accepted.

From the above review, it seems clear that several mechanistic pathways may exist that explain how periodontitis might be causally linked to CVD. Some or all of these proposed biological explanations most likely occur simultaneously and could be the direct or indirect consequence of the pathogenic microbiota in periodontal lesions. In addition, genetic variations in inflammatory pathways common to both diseases could indirectly explain some of the findings outlined in the current review.

In this review, we have summarized potential host response mechanisms to incident bacteremias. In addition to genetic variations within individuals (as discussed for *ANRIL*), we emphasize that individual host response mechanisms are also affected by population-specific differences related, for example, to ethnicity, dietary habits and nutritional availabilities and life style factors. These considerations make it difficult to generalize at this point potential host response mechanisms to might form the causal link between periodontitis and CVD.

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Clinical Relevance

Scientific rationale for the study: There is a need to determine whether inflammatory mechanisms explain the links between periodontitis and cardiovascular diseases (CVD).

Principal findings: Many studies show that periodontitis is associated with increased systemic inflammation and that a variety of mechanisms may account for these observations.

Treatment of periodontitis generally decreases levels of inflammatory mediators. Thus, it is possible that inflammation due to untreated periodontitis contributes to the development of inflammatory lesions in atheromatous plaques leading to CVD.

Practical implications: The relative degree of increased levels due to periodontitis of well-documented inflammatory risk markers such as C-reactive protein (CRP) is sufficient

to theoretically increase risk for CVD, but levels are only modestly, and possibly transiently, reduced by periodontal therapy. In addition, there are little data relating inflammatory factors to clinical end-points such as thrombotic events or myocardial infarction. Thus, it remains to be shown that this enhancement of systemic inflammation significantly affects risk for or development of CVD.