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SPECIAL REVIEW IN PERIODONTAL MEDICINE

The link between periodontal disease and cardiovascular disease is probably inflammation

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A periodontal–cardiovascular relationship has been postulated for several years but a definitive etiologic relationship has not been established. However, evidence continues to accumulate epidemiologic and otherwise that periodontitis at the very least plays a contributory role in the progression of cardiovascular disease. This paper will argue if indeed such a causative relationship exists, it is most likely based on the incremental contribution of chronic periodontal inflammation on systemic inflammation rather than the direct colonization of atherosclerotic plaques with periodontal pathogens.
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Background

A significant *albeit* modest association between cardiovascular disease (CVD)/stroke and periodontal disease has been established on the basis of epidemiologic studies in recent years (Janket *et al*, 2003; Scannapieco *et al*, 2003a,b). Whether or not this is an etiologic relationship, i.e., does periodontitis contribute to the pathogenesis of CVD/stroke is not yet well established. The question is an important one. In the United States, CVD accounts for about half the deaths each year, whereas periodontal disease affects as many as 75% of individuals (DeStefano *et al*, 1993; Beck *et al*, 1996) Traditional risk factors for CVD such as hypercholesterolemia, smoking, and hypertension explain about half the incidence of the disease (Futterman and Lemberg, 1998). In the case of periodontal disease, about half the incidence of periodontal disease can be attributed to genetic factors (Michalowicz *et al*, 2000). As investigators have sought to understand and elucidate unaccounted for risk factors, the role of inflammation in the pathogenesis of both diseases has taken on increased significance. Indeed Ross (1999) has described atherosclerosis, precursor to ischemic events involving the heart, brain and extremities as an inflammatory disease. Elevated markers for systemic inflammation are now considered recognized risk predictors of CVD (Pearson *et al*, 2003). Work conducted by our group and others has demonstrated the importance of altered inflammatory responses both to an individual's susceptibility to periodontal disease and to the destruction observed in periodontal disease (Kantarci *et al*, 2006). Furthermore, it has been shown that periodontal disease contributes to an increase in systemic inflammation (Glurich *et al*, 2002; Persson *et al*, 2005). Periodontal disease can usually be diagnosed and treated successfully although it often is not (Becker *et al*, 1983; Axelsson *et al*, 2004). However, the benefits of treating periodontal disease exist apart from the impact on CVD, and the side effects of treatment are few if any. If it is shown that periodontitis plays a causal or contributory role in the pathogenesis of CVD, a significant new therapeutic avenue will be opened to mitigating the risk of CVD.

This paper discusses in general terms the available epidemiological evidence and its relative importance. Studies done in animal models on the impact of the treatment of periodontal disease on secondary outcomes of CVD/stroke in humans are then reviewed in much more detail. It will argue that the collected weight of the evidence is compelling, if not conclusive according to Evidence Based Medicine (EBM) criteria, that a causal relationship exists. It will further argue that the most probable mechanism for linkage is inflammation and not directly infectious.

Epidemiologic evidence and its limitations

The Oxford Center of Evidence Based Medicine (CEBM; http://www.cebm.net/levels_of_evidence.asp) has established a hierarchy to be used in assigning

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Table 1 Levels of etiologic evidence

Level		*Criteria*
1	a	Systematic Review (SR) (with homogeneity of RCTs)
	b	Individual RCT (with narrow confidence interval)
	c	All or none
2	a	SR (with homogeneity) of cohort studies
	b	Individual cohort study (including low quality RCT; e.g., <80% follow-up)
	c	Individual cohort study (including low quality RCT; e.g., <80% follow-up)
3	a	SR (with homogeneity) of case
	b	Individual case
4		Case-series (and poor quality case–control and cohort studies)
5		Expert opinion without explicit critical appraisal, or based on physiology, bench research or "first principles"

weight to a particular study seeking to answer questions of cause and effect. At the top of this hierarchy sits the randomized controlled clinical trial, whereas at the bottom is evidence from animal studies, basic research, and expert opinion (Table 1). According to EBM principles, results of more than one randomized controlled clinical trial asking some form of the question 'Does controlling periodontal disease reduce the incidence of CVD/stroke?' would be required to definitively answer the question of causality as it relates to periodontal disease and CVD. However, for logistic, financial, and ethical reasons this is a not a simple undertaking, and to-date not a single such study has been published. While a pilot of such a study is currently underway and includes the participation of our group as one among many, the results will not be available in the near term.

Janket *et al* (2003) conducted a metanalysis of the available epidemiologic evidence up until February 2001 and found a relative risk of 1.19 for developing CVD/stroke in patients with periodontitis. This risk was elevated in individuals younger than 65 and the relative risk of stroke alone was 2.85. The available studies used were eight prospective cohort studies and one retrospective cohort study. As mentioned above, no Randomized Controlled Trial (RCT) was available. Therefore, according to EBM criteria the available evidence is level 2b. Beck and Offenbacher (2005) recently conducted a narrative review of the subject and concluded as have many others that RCTs were necessary to definitively answer the question of causality. Scannapieco *et al* (2003a,b) in a consensus report concluded that there was as yet insufficient evidence available to recommend periodontal intervention to reduce the risk of CVD/stroke.

Periodontal disease is usually a chronic inflammatory condition that develops over a number of years, but in most individuals does not manifest itself until the fourth or fifth decade of life. Likewise, Libby *et al* (1997) have made the point that incipient atheromatous lesions are detected as early as the second decade of life. Therefore, it is reasonable to argue that contributory effects of periodontal disease on CVD takes place over a similarly extended period of time. Simply treating periodontal disease may have minimal or no effect on the incidence of CVD as the damage will have accrued over time and be largely irreversible. Thus, interventional trials may not be the best way in which to establish a link between periodontal disease and CVD. Indeed, we would agree with Dietrich and Garcia (2005) that a preponderance of evidence from other studies – both epidemiological and basic research – may be sufficient to establish a link and plausible explanation for that link.

In their study, Dietrich and Garcia (2005) have conducted a critical appraisal of the value of evidence-based criteria when answering the question of etiology in general and specifically as it relates to periodontal disease and CVD/stroke. The authors point out that an RCT can only test the value of a particular intervention on a given outcome: for example, the value of scaling and root planning at 3-month intervals on the incidence of CVD in a test group relative to control population. Such a study would not definitively answer the question of whether periodontal disease contributes to CVD, but only whether there is a benefit to scaling and root planning every 3 months on the incidence of CVD. If the answer to that question were no, the argument could still be made that alternative periodontal treatments would be more efficacious or that damaging effects are cumulative and irreversible. Thus, RCTs by their very design may only be able to answer very narrow questions. Dietrich argues that the strict application of EBM criteria when answering questions of etiology may be misguided and study design alone should not be the exclusive criteria when assigning weight of evidence to a particular study.

Significant and important issues to be considered when evaluating a study include, although are not limited to disease classification, disease stratification, modality of treatment, and population under study. One of the most common problems cited by the authors of the Dietrich study is misclassification of periodontal disease. For example, the majority of the studies included in the Janket meta-analysis used various periodontal disease indexes that have been generally shown to have low sensitivity and thus underestimate the true prevalence of periodontal disease. This in turn would be expected to mitigate the impact of periodontal disease on CVD as determined by the study results. As an illustration of the value of lower levels of evidence Dietrich *et al* cite the fact that the role of tobacco use in the pathogenesis of periodontal disease was established on the basis of the collected weight of observational studies and not of RCTs. They make the point that an RCT to investigate such a relationship would likely have been unethical. Thus, it is possible that studies of lower value on the EBM scale may be of very high quality and provide very useful information.

Animal studies

As was mentioned above, CVD/stroke are increasingly thought of as an inflammatory process. Markers and/or mediators of systemic inflammation that are considered to be important in the pathogenesis of atherosclerosis include C-reactive protein (CRP), interleukin (IL)1β, IL-6, serum amyloid-A (SAA), fibrinogen, and tumor necrosis factor (TNF) (Ridker *et al*, 2000; Libby *et al*, 2002). Much of the work both in humans and animals have investigated the impact of periodontal disease and periodontal treatment on measured levels of these markers. In addition, some studies have investigated the impact on more traditional markers of CVD/stroke such as serum lipids and hypertension. The impact of periodontal infections on the dimensions of atherosclerotic lesions has been studied directly in mouse, rabbit, and minipig models.

Much of the proof of principle evidence in the relationship between periodontitis and CVD/stroke are based on animal studies. These investigations have taken the approach of inducing or simulating periodontal infection in a given animal model – usually mouse, rabbit, or pig – and observing the changes in dimensions of atherosclerotic lesions. In addition, these studies have gone the step further in investigating the effect of treatment of the periodontal disease on these same parameters.

Rabbit studies carried out by our group have clearly shown that at least in the rabbit, periodontal destruction is mediated primarily by the inflammatory response of the rabbit even though periodontal pathogens are a necessary etiologic agent (Jain *et al*, 2003; Serhan *et al*, 2003). Thus, we were able to induce periodontal disease in rabbits with the use of ligatures saturated with *Porphyromonas gingivalis* (Pg) while ligatures alone without Pg caused only very mild destruction (Jain *et al*, 2003; Serhan *et al*, 2003). The most significant aspect of these experiments was that periodontal destruction induced by Pg-soaked ligatures could be almost completely attenuated by the topical application of anti-inflammatory synthetic compounds called lipoxins-analogs. Transgenic rabbits over-expressing endogenous lipoxins exhibit an extremely low inflammatory phenotype. As expected, these animals showed minimal periodontal destruction in the presence of Pg-soaked ligatures (Serhan *et al*, 2003). We fed non-transgenic rabbits a high fat diet known to induce the formation of atherosclerotic lesions in their carotid arteries. At the same time, we induced periodontal disease using the Pg-soaked ligature model. The test group of non-transgenic rabbits received topical application of synthetic lipoxin analog whereas the control group did not. Again periodontal destruction was attenuated in the presence of endogenous or exogenous lipoxin Figure 1. Furthermore, the size of the atherosclerotic lesion found in the carotid artery was negatively correlated to the presence of lipoxins (Jain *et al*, 2003). Thus, at least in this rabbit model, periodontal destruction can be said to be bacterial in etiology but inflammatory in its pathogenesis. The formation of atherosclerotic plaques in these same rabbits may be induced by a high lipid diet, but the progression of the lesion is accelerated in the presence of periodontal destruction and prevention of inflammation results in smaller plaques Figure 2.

It should be noted that lipoxins have no known antimicrobial effect (Van Dyke and Serhan, 2003).Furthermore, Pg were not found within the body of the atherosclerotic plaque. It has been suggested that the linkage between periodontal disease and CVD/stroke is directly infectious, i.e., bacteria directly within the atheroma may cause or accelerate the development of the atherosclerotic plaque (Chiu, 1999). However, in the rabbit model this is not the case. The more likely

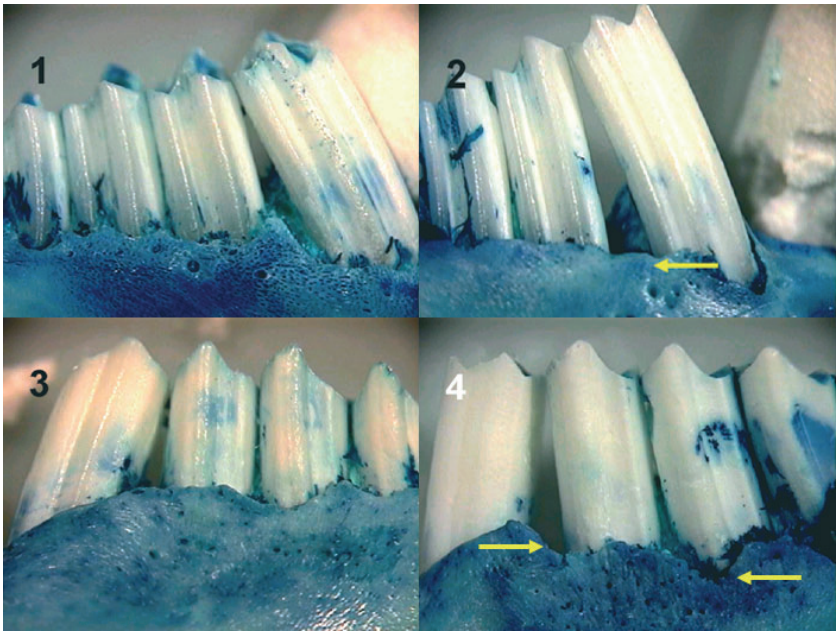

Figure 1 Delfeshed rabbit jaw specimens. Non-transgenic rabbit with Pg ligature and topical lipoxin (test) *1 and 3* and without topical lipoxin (control) *2 and 4*. Note that significantly less periodontal destruction was observed in test specimen versus control. Similar results to the test group were observed in transgenic animals over-expressing endogenous lipoxin

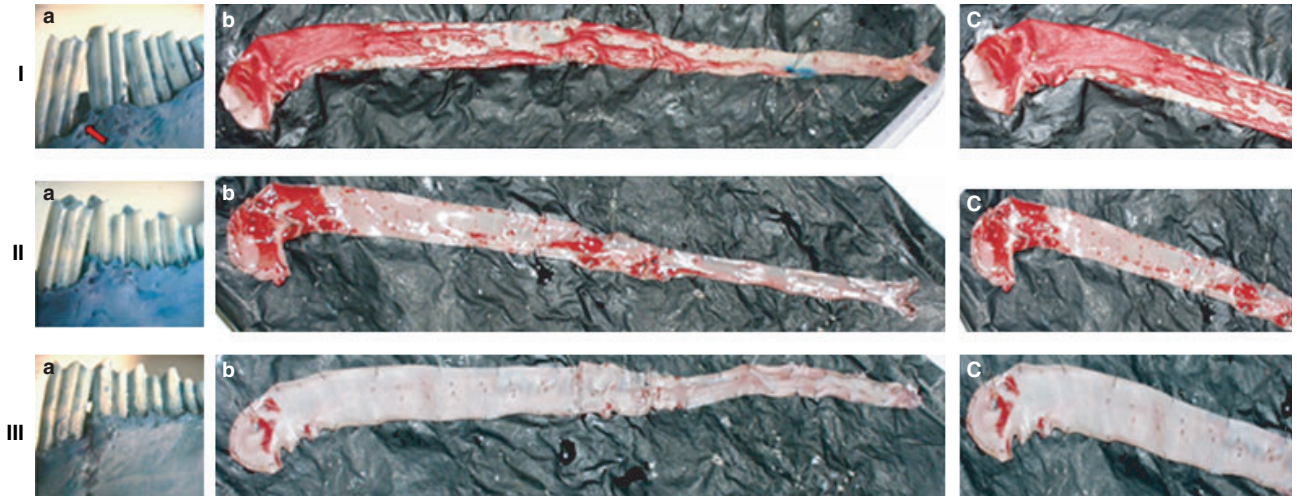

Figure 2 The impact of periodontitis on early atheromatous changes: Top- a normal animal (no periodontitis) on a high fat diet accumulates moderate lipid deposits in the aorta; Middle- an animal with ligature induced periodontitis on a high fat diet accumulates significantly more lipid in the aorta; Bottom- a 15-LO transgenic rabbit is resistant to both ligature induced periodontitis and high fat diet induced lipid accumulation. The 15-LO transgenic rabbit has increased levels of circulating lipoxins

connection is the host response to bacterial or other challenge. In two rabbits treated with Pg ligatures, periodontal disease failed to develop which is consistent with the observed variable susceptibility to periodontal disease in humans. Just as importantly, both of these rabbits failed to develop significant atheromatous lesions despite the high lipid diet (Jain *et al*, 2003). A reasonable explanation for these observations is that the innate immune response plays an important role in both the pathogenesis and progression of periodontal disease and CVDS/stroke.

A separate group of investigators have looked at the impact of challenge with periodontal pathogens specifically Pg-on markers of CVD/stroke and on atheromas themselves in apo-E mice. The apo-E mouse is a well-accepted model for the study of lipid-mediated CVD. Apo-E mice are genetically predisposed to high serum levels of cholesterol and thus the development of atheromatous lesions. Li *et al* (2002)investigated the effect of Pg on the progression of atheromatous lesions taken from the aorta of heterozygous Apo-E mice and wild type mice. The mice were divided into two groups, one fed a high fat diet and one a regular chow diet. Pg was administered *intravenously* at regular intervals. The animals were then killed and the atheromas dissected out and measured macroscopically and examined histologically. Atheromas were more developed in animals that were administered Pg than in those that were not. The investigators found elevations of systemic markers of inflammation such as CRP and SAA in animals injected with Pg, and Pg was localized to the lesions themselves suggesting that they may play an active and direct role in the development of these lesions. However, it should be noted that Pg were injected directly intravenously in relatively large concentrations and thus the Pg observed in these lesions could very likely be considered an artifact of the study design. Although the investigators concluded that the most likely explanation for the accelerated development of atheromatous lesions was direct bacterial colonization, the data could just as easily support the conclusion that the relationship is based on inflammation as elevations in serum levels of markers of systemic inflammation were observed. Intravenous injections of Pg in normocholesterolemic and hypercholestorlemic minipigs confirmed these results. Whether the bacteria induce elevation in systemic inflammation or induce inflammation locally, the fact remains that the cardiovascular lesion is an inflammatory lesion.

The same group investigated the effect of IL-1 ablation on the progression of atheromas in Pg-challenged Apo-E heterozygotes fed regular chow or high fat diet (Chi *et al*, 2004). The mice were also homozygous or heterozygous for the absence of the IL-1 receptor IL-1R1. IL-1 is a well-characterized proinflammatory molecule whose effects on target cells are mediated through its receptor IL-1R1. In this study, Pg was administered intravenously as above. The absence of IL1-R1 slowed the progression of atheromatous lesions in high fat diet animal and in animals treated with Pg. Thus blocking the proinflammatory actions of IL-1 played a central role in the progression of atherosclerotic lesions. The investigators did not discuss the presence or absence of Pg in the lesions.

Lalla *et al* (2003) orally inoculated Apo-E mice with Pg to better simulate periodontal infection. Control mice received no inoculation. They were able to demonstrate increased size of atherosclerotic lesions in infected mice as opposed to uninfected mice. A positive correlation was noted between the severity of periodontal bone loss in infected animals and the size of the atherosclerotic lesions. The study also showed elevations in serum Il-6 and increased endothelial expression of Vascular Cell Adhesion Molecule (VCAM-1) a key pro-inflammatory molecule in the early pathogenesis of atherosclerosis. Using PCR, the investigators demonstrated the presence of Pg DNA in the body of the atherosclerotic lesions.

Gibson *et al* (2004) have similarly shown that homozygous Apo-E mice fed a regular chow diet and challenged with topical oral and anal Pg showed accelerated development of atherosclerotic lesions relative to unchallenged controls. In the same group of studies, the investigators have also demonstrated the presence of Pg in the intravascular compartment using PCR. They have also demonstrated that Pg is able to upregulate the expression of pro-inflammatory molecules *in vitro* and that the ability of Pg to attach and invade endothelial cells was necessary for this to occur. The inflammatory response of endothelial cell as measured by the expression of pro-inflammatory cell surface molecules and soluble cytokine has been shown to be an early and necessary step in the formation of the atherosclerotic lesion. Thus, they make the argument that the actual presence of Pg in the intravascular compartment and intracellular within endothelial cells is necessary for the observed accelerated atherosclerotic phenomenon observed in the presence of Pg. It has to be noted that viable Pg were not noted either intravascularly or intracellularly in the *in vivo* model. A possible alternative explanation for the presence of Pg 16s ribosomal RNA within the intravascular compartment is recirculation of phagocytic cells.

Human studies

While there are no interventional trials directly investigating the impact of periodontal treatment on risk of CVD/stroke, a number of studies have examined correlations between periodontal disease and treatment of periodontal disease and traditional and emerging risk factors for CVD/stroke.

Traditional markers of CVD have included dyslipidemia, obesity, hypertension, cigarette smoking, diabetes mellitus, and metabolic syndrome. Emerging risk factors include homocysteine, atherogenic lipoprotein and CRP and fibrinogen (Pearson *et al*, 2003). The two latter indicators are reflective of levels of chronic low-grade systemic inflammation. As was mentioned above, atherosclerosis is now viewed in terms of a progressive inflammatory process rather than as simply an accumulation of lipids. In a case–control prospective cohort study examining serum markers of peripheral vascular disease, Ridker *et al* (2001)identified CRP as the most significant non-lipid predictor of CVD whereas total cholesterol (TC)-low density lipoprotein was the strongest lipid predictor. These were also the two strongest independent predictors of peripheral vascular disease. Furthermore, traditional risk factors including dyslipidemia can all be linked to inflammatory processes according to evidence reviewed in a recent article from Libby *et al* (2002).

D'Aiuto *et al* (2006)have examined the effect of non-surgical treatment of periodontal disease on serum CRP and IL-6 measured using a high sensitivity assay (hs-CRP) and ELISA, respectively. They conducted a prospective longitudinal single blind trial of otherwise healthy patients suffering from severe periodontal disease. Serum levels of CRP and IL-6 were measured prior to and up to 6 months after completing of subgingival scaling and root planning. Subgingival periodontal pathogens were assayed using PCR. Patients were also treated for any other primary dental disease requiring basic restorative, endodontic or oral surgical (extractions) treatment. This was completed prior to the completion of periodontal treatment. An untreated control group was not included on ethical grounds given the severity of the periodontal disease. At baseline, serum levels of CRP and IL-6 were 1.9 mg l^{-1} and 1.8 ng l^{-1}, respectively. Significant reductions between baseline and 6-month posttreatment were noted in both CRP and IL-6. Patients who responded better to periodontal therapy demonstrated greater reductions in levels of CRP and IL-6. These results remained significant after controlling for confounding variables including smoking, body mass index, age, and gender. In a second study using a similar design but testing two treatment regimens, the same group confirmed the above results and also demonstrated a short-term significant reduction in serum LDL and blood pressure (D'Aiuto *et al*, 2005). The principal limitations of the above studies were their small sample sizes of 94 and 40 individuals, respectively. The limited follow-up period of 6 months in both cases raises the issue of whether reductions could be maintained over a longer time period with ongoing periodontal treatment. Similar results with respect to CRP were noted by Mattila *et al* (2002) and Montebugnoli *et al* (2005) in short term prospective interventional studies utilizing 19 and 35 subjects, respectively.

Deliargyris *et al* (2004)in a case–control study design demonstrated that the incidence of periodontal disease was three times higher in a group of individuals who had suffered a myocardial infarction relative to a well-matched healthy control group. Furthermore, serum levels of CRP were higher in individuals who had suffered both an MI and periodontal disease *vs* those individuals who had suffered only one or the other. The study included statistical accounting for confounding factors including smoking, diabetes mellitus, and infarct size. In a cross-sectional study, Loos *et al* (2000)have confirmed the elevation of CRP and IL-6 in patients suffering from periodontal disease *vs* a matched healthy control group.

In a cross-sectional study design, Amar *et al* (2003) demonstrated higher levels of CRP in patients suffering from severe periodontal disease relative to matched healthy controls. In addition, they were able to demonstrate disrupted endothelial- and nitroglycerin-mediated brachial artery flow in test patients *vs* control. Seinost *et al* (2005)were able to demonstrate improvements in Doppler measured brachial artery flow following treatment of periodontal disease.

Cross sectional studies carried out by Pussinen *et al* (2005)have shown serological IgG to *Actinobacillus actinomycetemcomitans* and Pg were correlated with increased risk of coronary heart disease (CHD) in a Finnish population. Specifically they noted a twofold increase in risk for CHD after controlling for confounding factors.

Most recently a randomized controlled trial appearing in the New England Journal of Medicine from Tonetti *et al* (2007) revealed that intensive periodontal therapy results in an improvement in endothelial function after 6 months. Specifically after a course of subgingival scaling and root planning with adjunctive localized antibiotic therapy, plasma levels of soluble E-selectin were lower and flow-mediated dilatation higher in treated individual as compared with individual receiving only supragingival prophylaxis. Endothelial dysfunction has been shown to be associated with poorer prognoses in patients being treated for clinical coronary disease (Halcox *et al*, 2002; Fichtlscherer *et al*, 2004).

Aspects remaining to be elucidated

Studying the relationship between periodontal disease and CVD/stroke is complicated by a variety of factors. Both diseases share several risk factors including smoking, age, diabetes mellitus, and socioeconomic status. In addition, emerging evidence indicates that obesity and psychosocial stress, both risk factors for CVD/stroke, may also be risk factors for periodontal disease. The epidemiologic evidence to date shows a significant but modest relationship between periodontitis and CVD. Thus, controlled prospective trials with large sample sizes would ideally be carried out to tease out the true nature of the relationship if indeed one exists. However, for pragmatic and ethical reasons these studies are difficult to design, fund, and to complete.

It is now generally accepted that atherosclerosis is an inflammatory disease as opposed to a disease of excessive lipid accumulation. There is also growing consensus that tissue destruction observed in periodontal disease is more a function of the host inflammatory response as opposed to the destructive effects of the pathogenic organisms themselves. It has been suggested that chronic infections result in bacteremias that in turn colonize atherosclerotic plaques. Periodontal pathogens have been detected in atherosclerotic plaques in humans and in animal models (see above) using PCR techniques. As discussed above, *in vitro* experiments have demonstrated the ability of Pg to attach, invade and activate inflammation in endothelial cells. However, it must be said that viable bacteria have not been detected or colonized from atherosclerotic plaques. It may be that the nucleic acids that have been detected in atherosclerotic plaques are artifacts of study design or may have been trapped there *post hoc*.

It is the view of our group that much more compelling evidence suggests that if there is a link based on inflammation. Specifically we would argue that increases in systemic inflammation brought on by chronic infections result in up-regulation of inflammatory activity in endothelial cells that in turn results in accelerated development of atherosclerotic lesions. We believe the evidence from animal models carried out by our group and other groups is compelling in this regard.

Our group is also of the view that epidemiologic data obtained from human cross-sectional and longitudinal studies have established that periodontal disease results in an up-regulation of serum markers of inflammation, especially not only CRP but also soluble E-selectin. We are intrigued by recent longitudinal studies that suggest that controlling periodontal disease results in a reduction in the concentration of serum markers of inflammation and improved endothelia function. While molecular measures of inflammation and endothelial function are not routinely used in measuring risk for CVD or judging treatment outcomes, recent consensus statements published by the American Heart Association recognize their predictive value (Pearson *et al*, 2003). It is not yet clear according to the consensus statement whether or not therapies aimed at reducing systemic inflammation and endothelial dysfunction result in a reduced risk of CVD, but some studies that have been published do support this view (Pearson *et al*, 2003).

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