

Systemic Effects of Periodontal Diseases: Focus on Atherosclerosis

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1. Introduction

Periodontitis is a bacterially induced, localized, chronic inflammatory disease of periodontium that destroys connective tissues and bone supporting the teeth and may lead to tooth exfoliation and edentulism. Periodontitis is one of the most prevalent infectious diseases in humans. Mild forms affect 30% to 50% of adults and the severe generalized form affects 5% to 15% of the US adults (Periodontology, 2005). It is associated with specific bacterial groups, components of the dental biofilm, one of them (the “red complex”) closely related to clinical measures of periodontal disease (Socransky et al., 1998).

Periodontal pathogens normally inhabit the oral cavity as constituents of the dental biofilm. Since they are in intimate contact with the gingival epithelial tissues, they, however, can breach the gingival mucosal barrier at the ulcerative lesion and enter the circulation. Indeed, these organisms have been implicated in infections at distant sites, such as the central nervous system (Ewald et al., 2006), and measures of periodontitis (tooth loss) has been linked to subclinical atherosclerotic vascular disease (carotid artery plaque prevalence) (Desvarieux et al., 2003).

Systemic dissemination can be a result of tissue invasion, or of dental procedures including personal oral hygiene, leading to bacteremia. Tissue invasion is very likely a key virulence factor for a bacterium since it provides a “privileged niche” (Falkow, 1997) with access to host nutritional and iron substrates and a shelter from the host humoral and cellular immune response. Intracellular localization also brings about bacterial persistence, critical property of a causative agent of a chronic disease.

Atherosclerosis is a chronic inflammatory focal proliferative lesion of the arteries associated with conventional risk factors such as hypercholesterolemia, hypertension, diabetes and smoking, in addition to genetic factors (Libby and Theroux, 2005). However, the incidence of AS is not fully explained by these risk factors. It is now accepted that inflammation as a key integrative process playing a major role in the initiation and progression of atherosclerotic lesions, with the active participation of smooth muscle cells, leukocytes, growth factors and inflammatory mediators. A dynamic and progressive process, atherogenesis begins with endothelial dysfunction (“response to injury” model) that also interacts with the standard risk factors (Van Dyke and Kornman, 2008), (Libby et al., 2009).

Novel diagnostic and treatment modalities targeting vascular inflammation are dependent on further investigations of the origins of the inflammation. The focus of this review is the

contribution of periodontal pathogens to atherosclerotic inflammations, based on the latest communications.

Inflammation is involved in all stages of the atherosclerosis, from initiation through progression and, ultimately, the thrombotic complications (Libby et al., 2002). The role of inflammation as a direct causative factor in atherosclerotic vascular disease is intensely investigated (Libby et al., 2011). Thus, increased concentrations of high sensitivity C-reactive protein (hsCRP) have been shown to predict future acute myocardial infarction (MI) (de Beer et al., 1982). hsCRP levels were significantly elevated in 90% in unstable angina pectoris patients compared to 13% of stable angina patients; the average CRP values were significantly different ($p = 0.001$) for the unstable angina group (2.2 ± 2.9 mg/dl) compared to the stable angina (0.7 ± 0.2 mg/dl) groups (normal is less than 0.6 mg/dl) (Berk et al., 1990). Further, after adjustment for lipid and non-lipid factors, elevated CRP levels were significantly related to an increased risk of coronary heart disease (CHD), with relative risk 1.79 [at CRP levels ≥ 3.0 mg per liter, as compared with subjects with levels of <1.0 mg per liter (95% CI, 1.27 to 2.51; P for trend <0.001)] (Pai et al., 2004). Overall, the recognition of inflammatory character of atherosclerosis led to the successful application of hsCRP as acute-phase marker for cardiovascular risk assessment (Libby et al., 2010).

Response-to-injury model of atherosclerosis

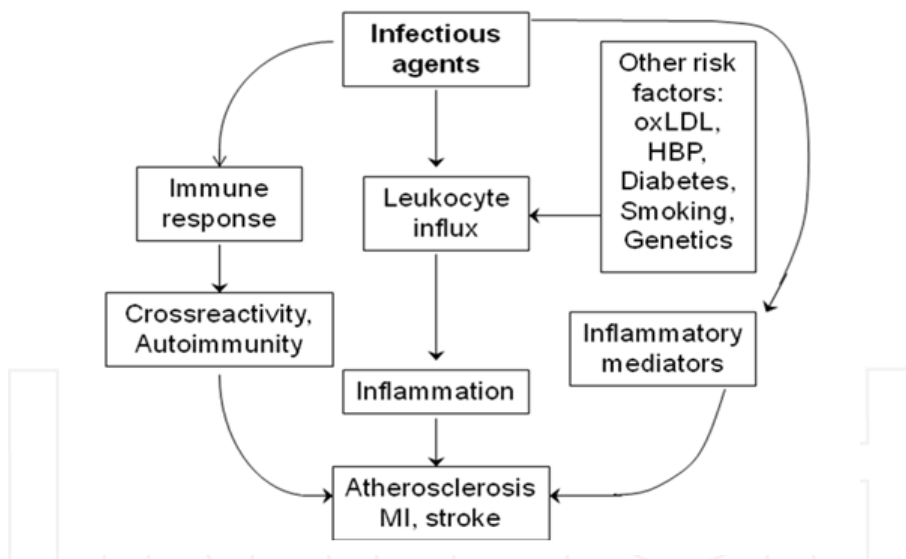


Fig. 1. Simplified chart representing the response to injury model of infectious agents-induced initiation and progression of the atherogenesis. Inflammation drives the initiation, progression, and eventually, the rupture of atherosclerotic plaques. The process involves inflammatory mediators and innate and adaptive immunity. A constant or repetitive injury may ultimately lead to necrosis, plaque rupture, myocardial infarction (MI) or stroke.

Pathogen burden. Since the incidence of atherosclerosis is only partially explained by the accepted risk factors, the attention was turned to infections as a potential cause of AS,

focusing on the total infectious burden (Ridker, 2002), (Epstein et al., 2009). The accumulated evidence suggests that the aggregate burden of the chronic infections, rather than a single pathogen, may contribute to increased risk of AS and clinical vascular events (Elkind, 2010). Indeed, an abundance of epidemiological evidence is presented in support of this notion (Ross, 1999), (Libby et al., 2002), and particularly in respect to periodontal infections [Desvarieux, 2005 #2905], (Demmer and Desvarieux, 2006), (Kebschull et al., 2010). The pathogen-initiated inflammatory process leading to endothelial cell activation, leukocyte rolling, adhesion and diapedesis, growth factor release, smooth muscle cell (SMC) proliferation and foam cell formation (Libby et al., 2010) all form the basis of the “response to injury” model of atherogenesis (Fig. 1).

Periodontitis as a risk factor for adverse ischemic events. Epidemiological and seroepidemiological studies addressed the association between these conditions relatively recently. The first **epidemiological** association was found between dental health and acute myocardial infarction (MI), where the former was significantly worse in 100 patients with MI than in 102 controls after adjustment for age, social class, smoking, serum lipid concentrations, and the presence of diabetes (Mattila et al., 1989). The results from the Oral Infections and Vascular Disease Epidemiology Study (INVEST) of 657 subjects with no history of stroke or myocardial infarction indicated that chronic infections, including periodontitis, may predispose to cardiovascular disease (CVD) (Desvarieux et al., 2005). In that study, mean carotid artery intima-media thickness (IMT) was related to the total bacterial burden, the periodontal bacterial burden, and to the relative predominance of periodontal over other bacteria in the subgingival plaque. After adjustments for age, race/ethnicity, gender, education, body mass index, smoking, diabetes, systolic blood pressure, and LDL and HDL cholesterol, it was demonstrated that periodontal bacterial burden was related to the carotid IMT, a measure of subclinical atherosclerosis ($P=0.002$). In other investigations, using a multivariate logistic regression model, periodontal bone loss was associated with a ~ 4-fold increase in risk for carotid atherosclerosis (adjusted OR, 3.64; CI, 1.37 to 9.65) (Engelbreton et al., 2005) and edentulousness was independently associated with the risk of aortic stenosis in a cohort of 2341 individuals (Volzke et al., 2005).

Using **seroepidemiology**, a study of 572 patients showed that the extent of atherosclerosis (using coronary angiography, carotid duplex sonography, and ankle-arm index) and CVD mortality were associated with elevated IgA and IgG titers to infectious agents. After adjustment for age, sex, classical risk factors and high hsCRP, infectious burden was significantly associated with advanced atherosclerosis, with an odds ratio (95% CI) of 1.8 (1.2 to 2.6) for 4 to 5 seropositivities ($P<0.01$) and 2.5 (1.2 to 5.1) for 6 to 8 seropositivities ($P<0.02$) (Espinola-Klein et al., 2002). Elevated levels of the periodontal pathogens *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* - specific serum IgG were associated with atherosclerosis (Colhoun et al., 2008). Interestingly, IgM antibodies specific for phosphorylcholine (PC), hapten-like epitope found on oxLDL and also on bacteria are atheroprotective; low PC-antibody titers are associated with an increased risk for CVD (Frostegard, 2010). Recently, in a study of 313 cases and 747 controls, using immunofluorescence microscopy and species-specific antibodies, the presence of six periodontal pathogens, *P. gingivalis*, *Tannerella forsythensis*, *Prevotella intermedia*, *Campylobacter recta*, *Fusobacterium nucleatum*, and *Eubacterium saburreum*, and their co-occurrence (0-6) was compared with the odds of having MI. Suggesting a role for a total

periodontal microbial burden, subjects with ≥ 3 periodontal pathogens species had about 2-fold increase in odds of having nonfatal MI than those who did not have any type of bacterial species [OR = 2.01 (1.31-3.08)], also suggesting that specifically the presence of *T. forsythensis* and *P. intermedia* was associated with increased odds of having MI (Andriankaja et al., 2011).

Interestingly, there is some noticeable discrepancy between the effects of periodontal infections on MI compared to ischemic stroke, even within the same populations and databases. While one such investigation found stronger association of periodontitis with stroke than with CHD (hazard rate 3.52; 95% confidence interval [CI], 1.59-7.81) (Jimenez et al., 2009), another study of 8032 subjects did not find "convincing evidence of a causal association between periodontal disease and CHD risk" (Hujoel et al., 2000). Still, using data from a total of 10,146 participants from the Third National Health and Nutrition Examination Survey (1988-1994), the link between periodontal health (gingival bleeding index, calculus index, and periodontal disease status, defined by pocket depth and attachment loss) and CVD risk factors (serum total and high density lipoprotein cholesterol, C-reactive protein, and plasma fibrinogen) was examined, showing a significant relation between indicators of poor periodontal status and increased CRP (Wu et al., 2000).

The results from these investigations vary significantly for variety of reasons, such as variations in study populations, differing measures - clinical (such as pocket depth and bleeding on probing) - and non-clinical (systemic antibody response or alveolar bone loss radiography) of periodontitis. These discrepancies suggest confounding factors common for periodontitis and CVD such as smoking that would interfere with the association between the conditions (Hujoel, 2002). Therefore, specific studies using multivariate and stratified analyses have been designed to address the confounding (Demmer and Desvarieux, 2006) and few meta-analyses have been published. One such analysis of PubMed, Cochrane Controlled Trials Register, EMBASE, and SCOPUS databases for references on periodontitis and CVD showed strong association between them, with a summary odds ratio of 1.75 (95% confidence interval (CI): 1.32 to 2.34; $P < 0.001$), compared to periodontally healthy subjects (Mustapha et al., 2007). Another meta-analysis of seven cohort studies supported the association and shows that periodontitis is a risk factor or marker for CHD, independent of traditional risk factors (Humphrey et al., 2008). Taken together, the epidemiological and seroepidemiological data suggest further investigation of the periodontal component of CVD.

Bacteremia. Since there are $10^8 - 10^{12}$ bacteria found per diseased periodontal site, large numbers of oral bacterial species, including periodontal can enter the circulation through the microvasculature following tooth brushing and other dental procedures (Iwai, 2009). Using PCR, hematogenous spread of bacteria was demonstrated in blood samples taken from 30 patients after ultrasonic scaling, periodontal probing and tooth brushing at 23%, 16% and 13% of the patients, respectively (Kinane et al., 2005). Further supporting the notion that periodontal organisms gain access to the circulation during dental hygiene procedures, another investigation of 194 patients demonstrated that periodontal site bleeding after tooth brushing was associated with ~8-fold increase in bacteremia (Lockhart et al., 2009). Unlike the clinical measures of periodontitis, the bleeding on probing was more associated with systemic inflammation than attachment loss (Beck and Offenbacher, 2002) and most associated with bacteremia (Lockhart et al., 2009).

2. Periodontal pathogen-accelerated endothelial injury and atherogenesis

The “response to injury” hypothesis presents atherosclerosis as an inflammatory disease, bearing similarity to a bacterial infection where the innate and adaptive arms of the immune systems are involved. In addition to the initiation and progression of the atheromas, inflammation is also related to the end stage of the disease, characterized by plaque rupture, atherothrombosis and acute ischemic events (Libby, 2007).

Several plausible models have emerged focusing on the pro-atherogenic mechanisms of action of periodontal pathogens. The immune response to periodontitis that may contribute to atherogenesis via pro-atherogenic systemic inflammatory response (immunological sounding) and autorecognition (autoimmunity, molecular mimicry) has been thoroughly reviewed elsewhere (Gibson et al., 2008), (Hayashi et al., 2010), (Teles and Wang, 2011).

Microbial invasion and its sequelae. In addition to immunological mechanisms, there are two metastatic avenues that periodontal organisms can exploit to reach vascular endothelia, direct invasion as a consequence of bacteremia and dissemination via internalization in migrating phagocytic cells (the “Trojan horse” approach).

Direct invasion. Periodontal bacteria have evolved elaborate strategies to invade non-professional phagocytes. Invasion of host cells is very likely a key virulence mechanism for a bacterium since intracellular residence provides “privileged niche” with 1) a nutrient-rich reducing environment with access to host protein and iron substrates, 2) partial protection from dental hygiene procedures including scaling and root planing (Johnson et al., 2008), 3) sequestration from the humoral and cellular immune responses, crucial at early stages of infection, 4) a means for replication and persistence that provides a reservoir and is essential for a chronic disease, and 5) protection from drug treatment (Eick and Pfister, 2004). Most available information regarding the invasive ability of periodontopathic bacteria concerns *P. gingivalis* [Lamont, 1995 #1268], (Dorn et al., 2001) and *A. actinomycetemcomitans* (Fives-Taylor et al., 1999), (Tomich et al., 2007). *Eikenella corrodens* and *Prevotella intermedia* were also shown to invade human primary endothelial and SM cells (Dorn et al., 1999). Collectively, it appears that the intracellular localization is a viable option for variety of periodontal pathogens (Tribble and Lamont, 2010).

Dissemination via internalization in migrating phagocytic cells (the “Trojan horse” approach). Unlike direct bacteremic dissemination, where bacteria spread in the circulation subject to opsonization and clearing by the humoral and cellular immune response, bacteria can metastasize after internalization in monocytes/macrophages or in dendritic cells (DCs) at the diseased site. Using such a “Trojan horse” approach, pathogens are able to disseminate and gain reach of endothelia, where due to extravasation of the carrier phagocytes they can localize at the activated endothelium in the arterial wall. A recently proposed model describes how *P. gingivalis* may exploit DCs to spread from the oral sites and gain access to systemic circulation. Thus, *P. gingivalis* may contribute to atherogenesis via subverting normal DC function, promoting a semimature, highly migratory, and immunosuppressive DC phenotype that contributes to the inflammatory development of atherosclerosis and, eventually, to plaque rupture (Zeituni et al., 2010). Supporting this suggestion, the infection with invasive *P. gingivalis* strain was shown to induce monocyte migration and significantly enhance the production of the pro-inflammatory cytokines (Pollreis et al., 2010).

Bacterial transmission between vascular endothelial and smooth muscle cells. In the only thorough investigation of *P. gingivalis* invasion of primary human endothelial and SM cells,

it was shown that the organism can spread intercellularly in vascular cell types (Li et al., 2008). This property has been previously demonstrated with a monoculture of gingival epithelial cells (Yilmaz et al., 2006). Using vascular cell cultures and immunofluorescence, the study demonstrates that bacteria can transmit between the same as well as between different cell types, from infected to fresh cells, leading to spreading of the infection that in clinical setting could lead to chronicity of disease (Li et al., 2008).

2.1 Proatherogenic consequences of bacterial presence in vascular cells

Activation of endothelial and smooth muscle cells. Endothelial cell activation is a pivotal moment in initiation of atherogenesis. It was shown that infection with *P. gingivalis*, but not with non-invasive non-fimbriated mutant induced the expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1) and P- and E-selectins in human endothelial cells (Khlgtian et al., 2002). Further, it was shown that *P. gingivalis* fimbria elicit chemokine production in human aortic endothelial cells via actin cytoskeletal rearrangements and that the pro-inflammatory IL-1 β , IL-8 and MCP-1 were induced in these cells (Takahashi et al., 2006). *P. gingivalis* infection activates host cells via TLR2 and TLR4 - mediated cell signaling (Hajishengallis et al., 2006), (Hayashi et al., 2010).

The smooth muscle cells (SMC) were found to respond to bacteria in a prothrombotic or in a proliferative manner (Roth et al., 2009), (Wada and Kamisaki, 2010). In the latter communication, it was shown that SMC proliferation in distal aorta aneurysms was associated with presence of *P. gingivalis* in the dental plaque of the patients.

Prothrombotic effects of bacteria and plaque rupture. An alternative mechanism through which bacteremias (or bacteria present in ruptured plaque) may contribute to vascular thrombosis is the triggering of the coagulation cascade (Herzberg et al., 2005), (Iwai, 2009). The potential adverse role of bacteria in atherothrombosis has been shown using human aortic SMC. Live invasive *P. gingivalis*, but not heat-killed or non-invasive mutant specifically suppressed tissue factor pathway inhibitor (TFPI) produced by vascular cells. The results suggested a procoagulant response of the host cells to bacteria (Roth et al., 2009). Plaque rupture, leading to exposure of the prothrombotic plaque core to the circulation and thrombus formation can be attributed to bacteria-dependent release of metalloproteinases (MMPs) with concomitant suppression of the MMP antagonist, tissue inhibitor of MMPs (TIMP) (Sato et al., 2009), (Guan et al., 2009).

Animal models furnish a useful research tool and are indispensable in testing hypotheses at a pre-clinical stage (Graves et al., 2008). In a study of wild-type *P. gingivalis* and a non-invasive FimA⁻ mutant, both strains were detected in blood and aortic tissue of ApoE^{-/-} mice by PCR after challenge, however only the invasive strain accelerated atherosclerosis in the animal model [Gibson, 2004 #2679]. Importantly, a prevention of *P. gingivalis*-accelerated atherosclerosis via immunization to control *P. gingivalis*-elicited periodontitis was demonstrated in the same study. Furthermore, using a mouse model of atherosclerosis and metronidazole administration followed by *P. gingivalis* i.v. inoculation, it was shown that 1) the lack of invasion ability of the mutant prevents the formation of aortic lesions in the animals inoculated with fimbriae-deficient strain (DPG3) compared to wild-type strain (381), and that 2) metronidazole, common antibacterial used in anaerobic periodontal infections, completely prevents the formation of *P. gingivalis* - associated arterial lesions (Amar et al., 2009). The results indicate that this oral pathogen can exert a critical damage on the vessels, and that drugs are viable treatment options. It further suggests that the bacterial

- endothelia interaction and activation causing phagocyte recruitment to the infection site may represent a key step in atherogenesis.

In addition, rabbit model with experimentally induced periodontitis developed fatty streaks in the aorta faster than in periodontally healthy animals, suggesting direct contribution of periodontitis to atherosclerosis (Jain et al., 2003) and recurrent *P. gingivalis* bacteremia induced aortic and coronary lesions in normocholesterolemic pigs and increases atherosclerosis in hypercholesterolemic pigs (Brodala et al., 2005). Altogether, variety of animal models have been used to demonstrate the adverse effect of periodontal bacteria on vascular health.

3. Association of periodontal bacteria with atheromata: Are we finally having “the smoking gun”?

Bacterial fingerprints in atheromas. As outlined above, there is strong evidence that oral bacteria can spread in the circulation during dental procedures such as tooth brushing (Lockhart et al., 2009). Since more than 700 bacterial species are identified in the mouth (Dewhirst et al., 2010), (Parahitiyawa et al., 2010), it is expected that many species, including *P. gingivalis* are disseminated to large vessels. Identification of the pathogens associated with atherosclerotic lesions can be performed using PCR and metagenomic approaches. Indeed, DNA from periodontal organisms, including *A. actinomycetemcomitans* and *P. gingivalis* were detected in atheromas by PCR [Haraszthy, 2000 #2237]. Using 16S rDNA PCR, it was also found that 1.5-2.2% of the total DNA in the atheromatous samples was bacterial, where large proportion of it was of oral origin, especially in the elderly group of individuals (mean age, 67 years). *P. gingivalis* was reported to be the most represented among the 10 species tested in this study (Kozarov et al., 2006), which is in line with its invasive properties described above (Dorn et al., 1999). It was also expected, since severe periodontal diseases (≥ 4 mm attachment loss) increase in prevalence with age (approximately 50% of 55-64 years old individuals have severe disease), and this is the age with the highest incidence of acute ischemic events.

Using clone libraries, in a comprehensive 16S rDNA PCR signatures study of atherosclerotic tissue from 38 CHD patients and 26 controls, bacterial DNA was found only in (all) CHD patients but not in controls. Presence of bacteria was confirmed by fluorescence in situ hybridization. A bacterial diversity of >50 different species was demonstrated, with a high mean bacterial diversity in atheromas, 12.33 +/- 3.81 (range, 5 to 22) (Ott et al., 2006). The broad spectrum of bacterial signatures encompassed species from the human barrier organs, the skin and the oral cavity.

Focus on causality. Cardiovascular disease is the leading cause of death and disability in industrialized countries. Although bacterial DNA has been recovered from atheromatous lesions and a link between inflammatory burden and atherogenesis has been established, there has been limited evidence that bacterial agents can be cultivated from atheromatous lesions. However, to fulfill Koch's postulate for infectious disease and to provide mechanistic data linking infectious agents to CVD, the cultivation of microorganisms from atheromatous tissue must be demonstrated.

Such cultivation has been eluding the biomedical community for decades. As a result (with the exception of *Chlamydomphila pneumoniae*), clinical strains could not be cultivated to provide key mechanistic link (Fiehn et al., 2005).

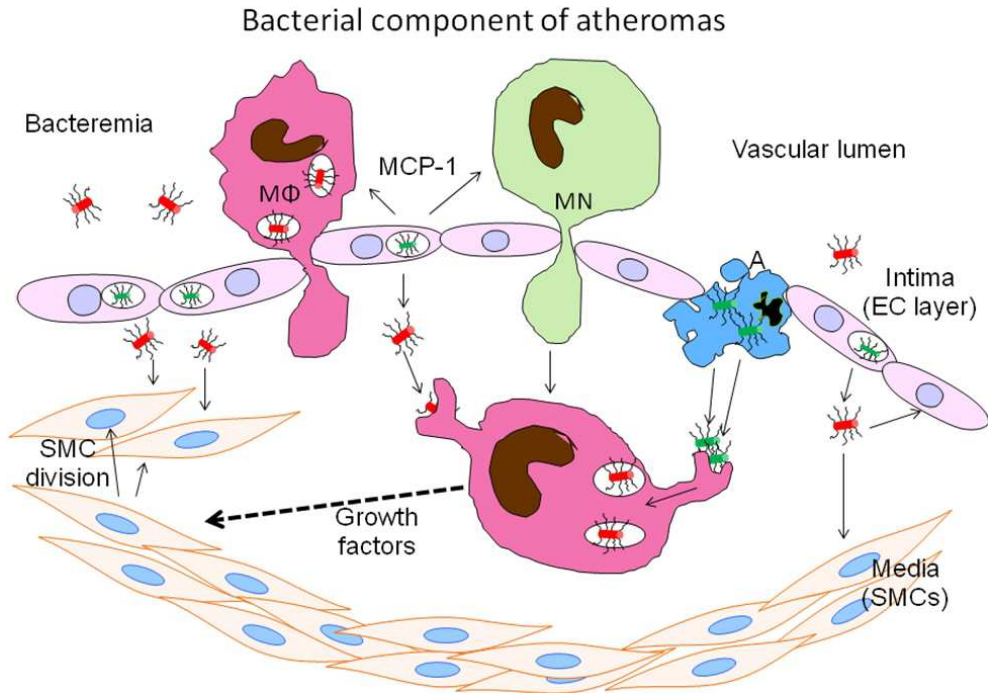
After viable *P. gingivalis* and *A. actinomycetemcomitans* in atheromatous vascular tissue were detected for the first time [Kozarov, 2005 #2700], bacterial transmission between primary vascular cell types was shown (Li et al., 2008) and finally, periodontal organisms including *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Streptococcus infantis* and *P. gingivalis* were recently cultivated from atheromatous tissue (Rafferty et al., 2011). In addition to *P. gingivalis*, a major periodontal pathogen, the identified species are no strangers to inflamed periodontium. *P. acnes* has been recovered from root canals and from blood samples taken during and after endodontic treatment (Debelian et al., 1998) and is the most prevalent species in apical periodontitis (Fujii et al., 2009). Importantly, using multivariable regression models, it has been shown that among patients with 25 or more teeth, those with two or more endodontic therapies had 1.62 times the odds (95% CI, 1.04-2.53) of prevalent coronary disease compared with those reporting never having had endodontic therapy (Caplan et al., 2009). *Staphylococcus* or *Streptococcus*, found in the oral cavity, are also detected in other systemic infections, in prosthetic valve endocarditis (Nataloni et al., 2010) and in heart valves and atheromas, respectively (Nakano et al., 2006), (Kozarov et al., 2006).

4. Atherosclerosis microbiome, the latest and most critical segment of the human microbiome may have a large periodontal component

Impact of genomics. Although multiple human microbiome projects have been launched, genomic studies of the atherosclerosis microbiome have not been initiated. Of note, out of 1,843 microbial genomes funded by the Human Microbiome Project by July 2011 (http://www.hmpdacc-resources.org/cgi-bin/hmp_catalog/main.cgi), none are associated with atherosclerosis. For comparison, 464 GI tract genomes have been selected for sequencing. A project targeting vascular inflammation-associated bacterial pathogens (the atherosclerosis microbiome) is conspicuously missing. This is simply due to the inability, until now, of the researchers to recover clinical isolates from diseased vascular tissue. Importantly, this segment of the human microbiome may represent a subset of the oral (and, possibly, gut) microbiome.

Focus on viable microbes. Identification of *viable* bacterial pathogens, members of the atherosclerosis microbiome, associated with human atheromatous tissue is critical for complete clarification of the potential for infectious etiologies of atherosclerosis and for reconsidering application of antibacterials in CVD treatment trials. Using a cellular immunology approach (Rafferty et al., 2011) allowing for cultivation of heretofore "uncultivable" bacteria from atheromatous tissue of vascular surgery patients, the community is now for the first time in a position to comprehensively address the bacterial component in vascular inflammations, the atherosclerosis microbiome.

With this approach, the identification of DNA from dead bacteria will be eliminated and only *bona fide* live organisms will be identified and investigated as the most likely targets for association with disease. The approach currently used, PCR of total atheroma DNA with species-specific primers or with universal primers followed by cloning of the amplification products and sequencing the resulting plasmid libraries (Ott et al., 2006) generates a large number of hits and is inconclusive. Bacterial DNA presence in the atheromata may be due to macrophages carrying their refuse, phagocytized bacteria from distant sites of the body, leading to many 16S rDNA bacterial signatures that may be false positives. The recovered by Rafferty et al isolates belong to four species only, both anaerobic and facultative. This approach will significantly decrease the complexity of the problem of false positives and obviate the need for investing in expensive or newly designed methods and equipment such as isolating a single bacterial cell from a specimen and sequencing its chromosome.



MN, monocyte. MΦ, macrophage with internalized bacteria. EC, endothelial cell. SMC, smooth muscle cell. A, apoptotic endothelial cell releasing intracellular bacteria.

Fig. 2. Bacterial infection-mediated model of atherogenesis presenting a bacteremic and macrophage-mediated tissue infection. Depicted are the tunica intima, a monolayer of endothelial cells (ECs) over a basal lamina that contains SMCs and the tunica media containing SMCs. Represented at left is the bacteremic microbial invasion of ECs. Within 24-72 hours the invading intracellular bacteria turn into non-cultivable state (in green). Endothelial activation is represented as release in the vascular lumen of proinflammatory mediators such as MCP-1. They activate circulating monocytes (MN) and macrophages (MΦ), promote their local adhesion and diapedesis into the lesion (in the center). MΦ can carry internalized persisting bacteria, thus contributing to the bacterial spreading. The activation of non-cultivable bacteria during vascular cell-cell transmission and the spreading of infection to adjacent ECs and to SMCs is shown at right and left. Additional bacteria are released in the atherosclerotic core following apoptosis and necrosis of host cells (at right). The phagocytosis of bacteria by a monocyte maturing into macrophage and the activation of dormant non-cultivable bacteria into active invasive stage (resuscitation, from green to red), following their ingestion is shown in the center. Growth factors released from the phagocytes promote SMC proliferation and migration (neointimal formation). For clarity, vasa vasorum neovascularization, lipids, foam cell formation, plaque rupture and blood coagulation/thrombus formation are not presented.

5. The heart of the matter: Current model of bacterial infection-accelerated atherogenesis, plaque rupture and acute ischemic events

A model of atherogenesis now emerges where (periodontal) bacteria invade endothelia either directly, following bacteremia, or are carried by phagocytes migrating from the primary infection site (the "Trojan horse" approach) (Figure 2). Upon invasion of endothelial cells, bacterial pathogens such as *P. gingivalis* are able to reside intracellularly for extended period of time, activating the endothelia and initiating the atherogenic process. Within 24-72 hours however, in order to sustain and persist, the bacteria switch into a dormant uncultivable stage (Li et al., 2008). Still facing a hostile environment (phagolysosomal fusion), some bacteria escape from the dormant stage, exiting into intracellular space and invading adjacent host cells, becoming transiently invasive and cultivable, and perpetuating persistent low-grade inflammation. The return to cultivable state specifically can occur after internalization by phagocytes (Rafferty et al., 2011). This leads to additional metastatic dissemination, injurious response, apoptosis and necrosis that are hallmarks of a chronic disease. Importantly, dormant bacteria have low metabolic activity, therefore targets for antibiotics are lacking and the organisms can become drug-tolerant. Using such mechanism, intracellular pathogens residing in atheromas could control their population yet allow for the observed persistent infection.

In conclusion, the epidemiological and seroepidemiological analyses, the in vitro and in vivo investigations, the presence in atheromata of live bacteria, some of them unique for periodontal lesions, and the clinical trials conducted so far, largely defend the argument that periodontal infections can be an exacerbating component of vascular inflammations. The latest data presented here expand the existing model of infectious component of atherosclerosis, identifying for the first time possible members of atherosclerosis microbiome, suggesting a novel mechanism for bacterial persistence in diseased tissue and for recurrence of disease and possibly explaining the failure of antibiotics to ameliorate the outcome after treatment of cardiovascular disease patients.

6. References

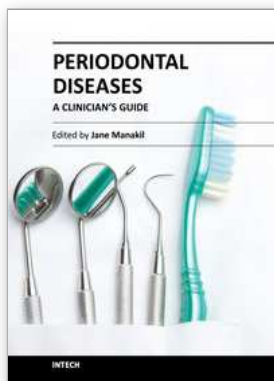
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"Periodontal diseases" is a web-based resource intended to reach the contemporary practitioners as well as educators and students in the field of periodontology. It is fully searchable and designed to enhance the learning experience. Within the book a description is presented of the current concepts presenting the complex interactions of microbial fingerprint, multiple genotypes, and host modulations. In addition, an overview is given of the clinical outcome of the disease's progression, as influenced by the epigenetic factors. Emerging concepts on periodontitis as a risk factor for various systemic diseases and as a bilateral modulating factor have been elucidated in detail as well.

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