Periodontal Disease, Atherosclerosis, Adverse Pregnancy Outcomes, and Head-and-Neck Cancer

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ABSTRACT

Interrelationships between periodontal infection and systemic conditions such as cardiovascular disease, adverse pregnancy outcomes, and head-and-neck cancer have become increasingly appreciated in recent years. Periodontitis is associated with cardiovascular disease (CVD) and, experimentally, with measures of atherosclerosis and endothelial dysfunction. Periodontal therapy may reduce atherosclerotic changes and improve endothelial function. Preliminary findings suggest a role for the genetic locus ANRIL in the pathobiology of both CVD and periodontitis. Periodontal pathogens induce anticardiolipin in periodontitis patients by molecular mimicry of the serum protein β-2 glycoprotein I. These antibodies have biological and pathological activities consistent with those reported for other infection-induced antiphospholipid antibodies. Anticardiolipin may explain some of the observed associations between periodontitis and systemic conditions such as CVD and adverse pregnancy outcomes. The oral commensal *Fusobacterium nucleatum* (*Fn*) becomes pathogenic on migration to extra-oral sites. *Fn* infection of the fetal-placental unit has been linked to pregnancy complications, including preterm birth, stillbirth, and early-onset neonatal sepsis. Reagents aimed at inhibiting or resolving inflammatory responses may be used to treat or prevent pregnancy complications due to bacterial infection. Chronic periodontitis may be independently associated with head-and-neck squamous cell carcinoma (HNSCC) through direct toxic effects of bacteria and their products, and/or through indirect effects of inflammation. Additionally, chronic periodontitis may facilitate the acquisition and persistence of oral HPV infection, a recently emerged risk factor for HNSCC.

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INTRODUCTION

The role of periodontal disease in the development and/or progression of systemic diseases and its potential contribution as a risk factor for such conditions have been the subject of much interest and investigation in recent years. Particular attention has been paid to the relationship between periodontitis and cardiovascular disease (CVD), given the high prevalence and the social and economic impact of cardiovascular events. There are also accumulating data supporting a possible relationship between maternal periodontal disease and adverse pregnancy outcomes.

Periodontal pathogens have long been implicated in the induction of systemic diseases, and ongoing research is revealing some of the direct and indirect mechanisms by which they are thought to induce or contribute to systemic disease processes.

In this article, evidence linking periodontal disease to systemic diseases, such as CVD and pregnancy complications, is described, and potential underlying pathogenic mechanisms are explored. The possible influence of periodontitis on the development of head-and-neck squamous cell carcinoma is also discussed.

Key Words

periodontitis, cardiovascular diseases, molecular mimicry, anticardiolipin antibodies, pregnancy complications, head-and-neck neoplasms.

PERIODONTITIS AND CARDIOVASCULAR DISEASE: SYSTEMIC, GENETIC, AND MECHANISTIC CONNECTIONS

Cardiovascular diseases (CVD) are caused mostly by atherosclerosis. Atherosclerosis typically leads to the highly prevalent myocardial infarction (MI) or the less prevalent cerebrovascular accident (stroke). Atherogenesis results in endothelial dysfunction, arterial stiffness, reduction of blood vessel lumen size, high blood pressure, and, ultimately, ischemic events. Preliminary findings implicate the possible involvement of the genetic locus ANRIL in the pathobiology of both CVD and periodontitis.

Periodontitis Is Associated with CVD

A strong association between periodontitis and CVD is supported by evidence from numerous cross-sectional and longitudinal studies. In 2009, the editors of the *American Journal of Cardiology* and the *Journal of Periodontology* wrote a joint consensus report and concluded that the association between periodontitis and cardiovascular disease really exists (Friedewald *et al.*, 2009). Evidence for how periodontitis could play an etiological role in CVD has been recently reviewed (Schenkein and Loos, 2013).

Measurement of Surrogate Markers of Atherosclerosis Associated with Periodontitis

The association between periodontitis and atherosclerosis has been evaluated by several indirect measures of atherosclerosis.

Periodontitis and Intima Media Thickness (IMT)

The IMT of the larger arteries that are relatively superficially positioned (*e.g.*, carotids, brachial arteries) can be measured non-invasively by ultrasound. An increase in IMT has been shown to predict cardiovascular events, and the IMT measurements of the carotid arteries are often used as surrogate clinical markers for the extent of atherosclerosis (Greenland *et al.*, 2010). The IMT of the carotid arteries in relation to periodontitis has been assessed in 19 studies, 11 of which found a higher IMT in periodontitis patients. After statistical adjustments for potential confounding factors, the reported association between periodontitis and increased IMT remained significant in nine studies.

Periodontitis and Flow-mediated Dilatation (FMD)

Flow-mediated dilatation (FMD) is a non-invasive atherosclerotic parameter evaluating endothelial function; it measures the % dilatation of the brachial artery in response to pharmacological and physiological stimuli (Anderson, 2006). Six case-control studies evaluated FMD in relation to periodontitis. Nearly all studies reported a significant endothelial dysfunction in periodontitis patients. However, a relatively large variation in endothelial function can be detected (2.35-12.4% reduced endothelium-dependent dilation [EDD] in individuals with periodontitis compared with control individuals).

Periodontitis and Pulse-wave Velocity (PWV)

Recently, arterial stiffness (AS), as assessed by pulse-wave velocity (PWV), has been examined to determine its relation to CVD. PWV is a reproducible non-invasive measure of large artery stiffness and has emerged as a novel biomarker for CVD (Graham *et al.*, 2007). AS represents a measure of whole arterial function, determined by both endothelial function and that of the intima, media, and adventitia of arteries. PWV has been established as a clinical parameter for the prediction of cardiovascular mortality and morbidity, independent of traditional cardiovascular risk factors (Vlachopoulos *et al.*, 2010).

Three cross-sectional studies determined PWV in systemically diseased individuals with and without periodontitis. No significant associations between periodontitis and PWV were found after adjustment for potential confounders. The prevalence of severe periodontitis was significantly higher in individuals with a PWV $\geq$ 14 m/sec, compared with those with a lower PWV. However, after adjustment for potential confounding factors, no significant difference remained. Therefore, to date, no clear association has been observed between periodontitis and PWV.

Periodontal Intervention and Arterial Function

Interestingly, non-surgical periodontal treatment has been shown to improve arterial function and/or reduce severity of atherosclerosis (Tables 1 and 2). Two studies noted no difference in IMT between baseline and 3 mos after periodontal therapy (Seinost *et al.*, 2005; Piconi *et al.*, 2009). However, a longer follow-up of 6 and 12 mos revealed a significant reduction in IMT compared with baseline (Piconi *et al.*, 2009). Thus, it is likely that the regeneration rate in the intima-medial layer is relatively slow and is clinically detectable only 6 mos after intervention.

Six studies utilizing FMD to measure endothelial function suggested a positive effect of periodontal therapy on FMD at 4 to 28 post-operative wks (see Tables 1 and 2). Tonetti *et al.* (2007) observed a significant improvement of the FMD at 6 mos, but not at 2 mos, after therapy; moreover, the FMD at 6 mos was significantly better than the FMD in those in the control group with periodontitis who obtained only community dental care.

A Common Genetic Risk Factor for CVD and Periodontitis?

Complex diseases like CVD, type 2 diabetes, and Crohn's disease may have similar and overlapping common causative genetic variants (Sivakumaran *et al.*, 2011); this is termed 'pleiotropy of complex diseases'. The ANRIL locus is the best-replicated coronary heart disease (CHD)-associated risk locus to date (Schunkert *et al.*, 2011). A highly increased risk for aggressive periodontitis (AgP) and limited evidence for increased risk with chronic periodontitis (CP) were observed with genetic

Table 1. Studies Evaluating the Effect of Non-surgical Periodontal Therapy on the Cardiovascular and/or Endothelial Function System in Individuals with Periodontitis: Duration, Population, and Periodontal Measures (relevant studies identified by literature review)

	Study Duration	Study Population	Periodontal Definition
Mercanoglu *et al.*, 2004	6 wks	28; systemically healthy	CAL, PPD, GI, PI
Seinost *et al.*, 2005	3 mos	30; systemically healthy	CAL, PPD, GI, PI
Blum *et al.*, 2007	3 mos	13; systemically healthy	CAL, PPD, BOP
Elter *et al.*, 2006	1 mo	22; systemically healthy	CAL, PPD
Li *et al.*, 2011	3 mos	25; systemically healthy	CAL and PPD (interproximal), BOP, PI
Tonetti *et al.*, 2007	6 mos	59; systemically healthy	PPD
Higashi *et al.*, 2008	6 mos	32; systemically healthy	Self-reported questionnaire
Higashi *et al.*, 2008	6 mos	26; individuals with hypertension	Self-reported questionnaire
Higashi *et al.*, 2009	6 mos	48; individuals with CVD	Self-reported questionnaire
Piconi *et al.*, 2009	12 mos	35; systemically healthy	PSR

CVD, cardiovascular disease; CAL, clinical attachment level; PPD, probing pocket depth; GI, gingival index; PI, plaque index; BOP, bleeding upon probing; PSR, Periodontal Screening and Recording.

Table 2. Studies Evaluating the Effect of Non-surgical Periodontal Therapy on the Cardiovascular and/or Endothelial Function System in Individuals with Periodontitis: Changes in the Vessel Wall as Measured by Intima Media Thickness (IMT) and Endothelial Function as Measured by Flow-mediated Dilatation (FMD) or Endothelium-dependent Digital Pulse Amplitude Testing (EndoPAT) (relevant studies identified by literature review)

Reference	Atherosclerotic Measure	Study Outcome		Conclusion /Significance
		Baseline	Final	
Mercanoglu *et al.*, 2004	FMD	Diameter: 3.9 ± 0.4 mm; EDD: 8.4 ± 4.0% EID: 13.3 ± 6.3%	Diameter: 3.8 ± 0.5 mm; EDD: 17.7 ± 5.7% EID: 24.9 ± 7.3%	NS $p < .0001$ $p < .0001$
Seinost *et al.*, 2005	FMD	Diameter: 3.9 ± 0.4 mm; EDD: 6.1 ± 4.4% EID: 13.3 ± 6.3%	Diameter: 3.9 ± 0.4 mm; EDD: 8.7 ± 5.7% EID: 13.3 ± 6.3%	NS $p = .003$ NS
	IMT	m-IMT (A. brachialis): 0.27 ± 0.07 mm	m-IMT (A. brachialis): 0.27 ± 0.06 mm	NS
Blum *et al.*, 2007	FMD	EDD: 4.12 ± 3.96% EID: 20.97 ± 10.7%	EDD: 11.12 ± 7.22 EID: 17.94 ± 6.23	$p = .0007$ NS
Elter *et al.*, 2006	FMD	EDD: 8.6 ± 4.7% EID: 19.8 ± 4.7%	EDD: 10.2 ± 3.9% EID: 21.3 ± 8.0%	$p = .034$ NS
Li *et al.*, 2011	Endo-PAT	2.41 ± 0.71	2.22 ± 0.62	NS; Adj. calculations for the treatment effect, $p = .03$
Tonetti *et al.*, 2007	FMD	#	Diameter changes: 0.06 ± 0.15 mm EDD changes: 1.48 ± 0.8% EID changes: –0.07 ± 2.44%	NS $p < .001$ NS
Higashi *et al.*, 2008	FMD	Diameter: 5.2 ± 1.3 mL/min EDD: # EID: #	Diameter: 5.4 ± 1.4 mL/min EDD: # EID: #	NS $p < .001$ NS
Higashi *et al.*, 2008	FMD	EDD: # EID: #	EDD: # EID: #	$p < .001$ NS
Higashi *et al.*, 2009	FMD	EDD: # EID: #	EDD: # EID: #	$p < .001$ NS
Piconi *et al.*, 2009	IMT	Bifurcation: 0.55 ± 0.03 mm 1-cm: 0.49 ± 0.02 2-cm: 0.5 ± 0.02	Bifurcation: 0.45 ± 0.04 mm 1-cm: 0.37 ± 0.03 mm 2-cm: 0.39 ± 0.03 mm	 $p = .01$ $p < .001$ $p = .001$

# Indicates not recorded. IMT, intima-media thickness; FMD, flow-mediated dilatation; EndoPAT, endothelium-dependent digital pulse amplitude testing; EDD, endothelium-dependent dilatation; EID, endothelium-independent dilatation; NS, not significant; a, arteria; Adj, statistically adjusted for potential confounding factors.

Table 3. Results from Genotyping of Independent Case-Control Population for Various Single-nucleotide Polymorphisms (SNP) in the *ANRIL* Locus (Schaefer *et al.*, 2009, 2011)

Type of Periodontitis	Ethnicity	N Patients	N Controls	SNP *p* value	Odds Ratio
G-AgP	German	151	736	6.9×10^{-4}	1.99
L-AgP	German	137	368	2.6×10^{-2}	1.72
G and L-AgP	Dutch	164	421	7.0×10^{-3}	2.53
G and L-AgP	German	301	962	4.0×10^{-4}	1.48
CP	Dutch	154	421	4.0×10^{-3}	0.57
CP	German	740	962	2.5×10^{-2}	0.66

Abbreviations: N, number; SNP, single-nucleotide polymorphism; G-AgP, generalized aggressive periodontitis; L-AgP, localized aggressive periodontitis; CP, chronic periodontitis.

variants in the ANRIL locus (Table 3) (Schaefer *et al.*, 2009, 2011). We speculate that there are likely to be common pathophysiologic pathways for both CVD and periodontitis.

ANTIPHOSPHOLIPIDS AND MOLECULAR MIMICRY: A LINK BETWEEN PERIODONTITIS AND SYSTEMIC DISEASE?

It is well-known that micro-organisms can produce pathology due to the phenomenon known as "molecular mimicry". Many microbes bear molecular structures of sufficient similarity to human tissue components so as to induce an immune response that is cross-reactive with human tissue. A group of autoantibodies termed "antiphospholipids" is related to pathology present in the Antiphospholipid Syndrome (APS) (Mehdi *et al.*, 2010). Patients who develop APS have greatly increased risk of thrombosis, fetal loss, and possibly early atherosclerosis. Analysis of data linking periodontitis to stroke, myocardial infarction, adverse pregnancy outcomes, and atherosclerosis prompted interest in these antibodies. In particular, anticardiolipin (anti-Cl) appeared to be of potential relevance to periodontitis, for two reasons. First, it was shown that monoclonal antibodies raised against the serum protein beta-2 glycoprotein I (β2GPI), which contains the target antigen for pathogenic anti-Cl (the peptide sequence TLRVYK), could induce APS-like pathology in mice (Bakimer *et al.*, 1992); and second, it was demonstrated that a variety of microbial pathogens could induce anti-Cl-like antibodies because they contained antigenic epitopes similar to TLRVYK in β2GPI (Blank *et al.*, 2002). These antibodies also produced APS-like symptoms in animal models.

Utilizing a commercially available ELISA kit, investigators examined serum samples from periodontally characterized patients for anti-Cl (Schenkein *et al.*, 2003). The proportion of chronic periodontitis (CP) and generalized aggressive periodontitis (GAP) patients testing positive for anti-Cl was significantly higher than that in periodontally healthy individuals (16-19% *vs.* 6.8%, $p = .0033$). It was hypothesized that the presence of these antibodies could be due to molecular mimicry of the TLRVYK peptide sequence of β2GPI by periodontal bacteria. Examination of the Swiss-prot database revealed a peptide sequence in the arg-gingipain (RGP) of *P. gingivalis* with homology to TLRVYK. When anti-Cl positive sera were absorbed from periodontitis patients with various strains of *P. gingivalis*, it was noted that all strains other than an RGP-defective mutant could remove most of the anti-Cl antibody from serum (Schenkein, 2005). Other investigators have found sequence homologies, and mutual cross-reactivity, between peptide sequences in both *Aggregatibacter actinomycetemcomitans* and *Treponema denticola* and β2GPI as well (Wang *et al.*, 2008; Chen *et al.*, 2009). Thus, elevated levels of anti-Cl in sera from periodontitis patients could very well be induced by periodontal pathogenic micro-organisms.

The ability of *P. gingivalis* and *A. actinomycetemcomitans* to induce anti-Cl was subsequently assessed in rabbits and mice. It was found that immunization of rabbits with *P. gingivalis* induced anti-Cl. Furthermore, affinity purification of anti-*P. gingivalis* antisera with either *P. gingivalis* or *A. actinomycetemcomitans* resulted in an approximately 40-fold purification of anti-Cl regardless of which bacterium was used. Some strains of *A. actinomycetemcomitans* were also observed to induce elevated anti-Cl in mice. Thus, it is clear that some periodontal pathogens can induce anti-Cl.

Effects of Anti-Cl on Human Endothelial Cell Activation

We have taken two approaches to assessing the biological activities of human anti-Cl in periodontitis patients. First, associations between elevated levels of anti-Cl and soluble markers of endothelial cell activation ICAM-1, VCAM-1, and E-selectin in serum were studied (Schenkein *et al.*, 2007). In a study of 290 periodontally characterized patients, it was observed that sera with elevated anti-Cl also contained elevated soluble markers of endothelial cell activation. This was especially apparent in patients with GAP and was observed whether or not the patients were current smokers. The individuals with elevated anti-Cl also demonstrated significantly elevated serum levels of C-reactive protein. Second, IgG was purified from sera of 53 individuals who were periodontally healthy or who were diagnosed with CP or GAP. These IgG preparations were added to cultures of human umbilical vein endothelial cells (HUVEC), and production of monocyte chemotactic protein-1 (MCP-1) was assessed. There was a strong correlation between anti-Cl titer and MCP-1 production ($p < .0001$, $r^2 = .48$), with higher MCP-1 production when titers of anti-Cl were elevated, regardless of clinical diagnoses. Furthermore, reduction of the anti-Cl content of the IgG preparations resulted in significantly decreased production

of MCP-1 by patient IgG (Schenkein *et al.*, 2013b). Thus, it appears that anti-Cl is biologically active, affecting endothelial cell function.

Effects of Anti-Cl on Pregnancy Outcomes in a Mouse Model

Experiments have also been undertaken to examine the effect of anti-Cl induced by *P. gingivalis* on pregnancy outcomes in a mouse model. This involved preparation of a series of mouse antisera to antigens that included β2GPI, *P. gingivalis* strain W83, and *P. gingivalis* strain HF 18 (an RGP-defective mutant of *P. gingivalis* strain W83). The results showed that anti-Cl titers were increased in sera from mice immunized with periodontal pathogens, other than the *P. gingivalis* strain (HF18) lacking the arg-gingipain protease (Table 4). Anti-Cl was then removed from these preparations by immuno-absorption on affinity columns containing cardiolipin complexed with β2GPI. Antibodies to the *P. gingivalis* strains were passively administered to mated mice at day 0 of pregnancy, and fetuses were harvested and evaluated at day 15. It was observed that anti-*P. gingivalis* induced fetal resorption at rates equivalent to that induced by anti-β2GPI, and that reduction of anti-Cl content of the antibody preparations resulted in a proportional reduction in fetal resorption. Furthermore, the RGP-defective mutant of *P. gingivalis* (strain HF18) failed to induce fetal loss, consistent with the presence of the cross-reactive epitope being present on the arg-gingipain protease (Schenkein *et al.*, 2013a).

Table 4. Relative Anti-cardiolipin Titers in Mouse Sera following Immunization

Antigen	IgG Anti-Cl Titer ± SEM[a]
β2GPI	2,438 ± 514
P. gingivalis W83	2,228 ± 283
P. gingivalis HF18[b]	228 ± 68
A. actinomycetemcomitans DB03A[c]	3,176 ± 93
A. actinomycetemcomitans DO45D[c]	1,313 ± 59
Alum alone	290 ± 183

[a]Titer was calculated as the inverse of the dilution of antibody for which OD = 1.0 in ELISA assay. Titers represent mean value from 5 antibody preparations

[b]*P. gingivalis* HF18 is an arg-gingipain defective mutant of *P. gingivalis* W83.

[c]Both strains of *A. actinomycetemcomitans* are clinical isolates from individuals with aggressive periodontitis.

PERIODONTITIS AND ADVERSE PREGNANCY OUTCOMES

Fusobacterium nucleatum: a Commensal Turned Pathogen

Increasing evidence suggests that oral bacteria can enter the systemic circulation under certain circumstances. Members of the oral microbiome can migrate away from the mouth, causing infections and inflammation at extra-oral sites (Han and Wang, 2013). *Fusobacterium nucleatum*, a Gram-negative common oral anaerobe, is one such "mobile" micro-organism. As an opportunistic oral commensal, *F. nucleatum* exists in both periodontally healthy and diseased sites. It is a heterogeneous species, with 5 established subspecies (subsp): subsp *animalis*, subsp *fusiforme*, subsp *nucleatum*, subsp *polymorphum*, and subsp *vincentii*. All 5 subspecies are present in the oral cavity. Outside the oral cavity, *Fn* becomes a "*bona fide*" pathogen, having been isolated from infections and abscesses from a wide range of organs and tissues, including liver, spleen, lung, kidney, blood, brain, and obstetrical and intestinal sites (Han and Wang, 2013). This section focuses on *F. nucleatum* infection in the intra-uterine cavity and its role in adverse pregnancy outcomes.

F. nucleatum and Pregnancy Complications

A series of clinical studies has linked *F. nucleatum* to a plethora of pregnancy complications, including preterm birth, stillbirth, and early-onset neonatal sepsis. By 16S rRNA gene-based PCR, *F. nucleatum* was found to be a highly prevalent species in amniotic fluid associated with preterm birth, while no microbial DNA was detected in normal pregnancies (Han *et al.*, 2009). A term stillbirth case in which *F. nucleatum* was isolated as a pure culture from the infant's lung and stomach was reported (Han *et al.*, 2010). An *F. nucleatum* clone identical to that from the stillborn infant was detected in the subgingival plaque of the mother, who had pregnancy-associated gingivitis with frequent gingival bleeding during gestation. It is possible that *F. nucleatum* translocated hematogenously from the mother's oral cavity to her uterus as a result of frequent dental bacteremia. Recently, it was reported that *Fn* was often concurrently detected in paired amniotic fluid and cord blood associated with early-onset neonatal sepsis (Wang *et al.*, 2013). From these studies and others, it was discovered that intra-uterine *Fn* infection is dominated by subsp *animalis*, followed by subsp *polymorphum* (Han and Wang, 2013).

Mechanism of *Fn* in Intra-uterine Infection

The mechanism of *F. nucleatum* in intra-uterine infection has been investigated in a pregnant mouse model. It has been shown that hematogenous injection of *F. nucleatum* (to mimic dental bacteremia) induces preterm and term fetal death in a dose-dependent manner, while injection of *E. coli* does not cause fetal loss (Han *et al.*, 2004). The pattern of infection resembles chorioamnionitis in humans (Han *et al.*, 2004, 2010). *F. nucleatum* colonize and proliferate specifically in the fetal-placental unit after crossing the endothelium (Han *et al.*, 2004). *F. nucleatum* colonization is dependent on its unique FadA adhesin (Ikegami *et al.*, 2009), a previously identified surface protein (Han *et al.*, 2005; Xu *et al.*, 2007; Nithianantham *et al.*, 2009; Han, 2011; Temoin *et al.*, 2012). FadA exists in two forms: the intact pre-FadA, consisting of 129 amino acids; and the secreted mature FadA (mFadA), consisting of 111 amino acids (Han *et al.*, 2005). Both forms are required for the formation of the active complex, FadAc, for binding and invasion of host cells (Xu *et al.*, 2007; Temoin *et al.*, 2012). FadA binding to VE-cadherin on the endothelial cells causes translocation of the latter to intracellular compartments and loosens the cell-cell junction, allowing bacteria in the vicinity, such as *E. coli*, to percolate through

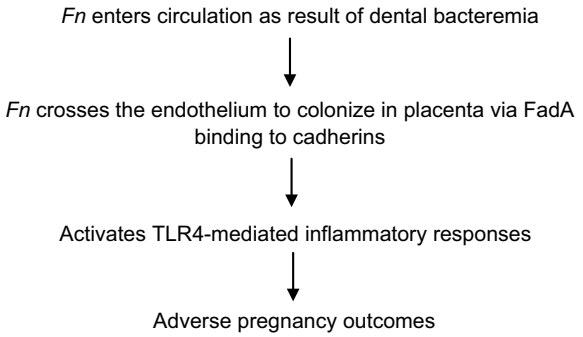

Figure 1. A model of oral *Fn* in induction of adverse pregnancy outcomes.

the endothelium (Fardini *et al.*, 2011). *F. nucleatum* crosses the endothelium either by direct invasion into the endothelial cells or through permeabilized cell junctions, and both mechanisms may be utilized for its hematogenous dissemination from the oral cavity.

Following colonization in the fetal-placental unit, *F. nucleatum* induces TLR4-mediated inflammatory responses (Liu *et al.*, 2007). In TLR4$^{-/-}$ mice or in wild-type mice treated with a TLR4 antagonist, *F. nucleatum* colonizes the placenta without inducing inflammation, leading to significantly improved fetal survival (Liu *et al.*, 2007). These observations suggest that reagents aimed at inhibiting or resolving inflammatory responses may be used to treat or prevent pregnancy complications due to bacterial infection. A model of oral *Fn* inducing adverse pregnancy outcomes is illustrated in Fig. 1.

PERIODONTITIS AND HEAD-AND-NECK CANCER

The epidemiology of head-and-neck squamous cell carcinoma (HNSCC) has changed in an unpredictable way over the last 3 decades. A steady increase in the incidence of oropharyngeal cancers has been observed in many parts of the world since the early 1970s, in spite of advances in prevention and treatment (Gillison *et al.*, 2012). A better understanding of HNSCC etiology, interactions among risk factors, and new approaches to prevention and treatment are necessary to change the course of this disease.

Chronic Periodontitis and Head-and-Neck Cancer

Clinical, epidemiologic, and animal studies have established a strong association between chronic inflammation and cancer of several organs (Kipanyula *et al.*, 2013). In the oral cavity, periodontitis is a chronic inflammatory disease associated with Gram-negative anaerobic dental plaque bacteria that promote the continuous release of bacteria and inflammatory cytokines into saliva (Scannapieco *et al.*, 2007). The prevalence of periodontitis in the general population is estimated to be 47% (Eke *et al.*, 2012).

It has been observed that chronic periodontitis was associated with increased risks of potentially malignant disorders (Tezal *et al.*, 2005) and HNSCC (Tezal *et al.*, 2007, 2009a). The strength of the association was greatest in the oral cavity, followed by the oropharynx and larynx. In addition, a history of periodontitis predicted poorly differentiated tumor status in patients with cancer of the oral cavity. An unexpected finding was that the association between periodontitis and HNSCC was weaker in current smokers compared with former and never-smokers. Supporting these results, other studies have also reported that the associations of oral health variables with head-and-neck, esophageal, upper gastrointestinal, and pancreatic cancers were weaker in smokers compared with non-smokers (Abnet *et al.*, 2005; Michaud *et al.*, 2007; Hiraki *et al.*, 2008). These observations, seemingly paradoxical, are consistent with the biological effects of tobacco smoke, which causes acute vasoconstriction and inhibits angiogenesis, proliferation, and production of inflammatory mediators (Laan *et al.*, 2004). However, these potent suppressive effects of smoking are reversible within a few hours of cessation. It was shown that smoking one cigarette every 2 hours inhibited the LPS-induced production of inflammatory cytokines in bronchial epithelial cells (Laan *et al.*, 2004). Clinical signs of gingival inflammation also increase after smoking cessation (Nair *et al.*, 2003). It is thus possible that while multiple toxic components can initiate carcinogenesis, other components in tobacco may delay clinical manifestations. Therefore, treatment of sources of inflammation in the oral cavity before smoking cessation should be an important component of both smoking cessation and cancer management.

Chronic Periodontitis-HPV Synergy in Head-and-Neck Cancers

Oral human papillomavirus (HPV) infection has emerged as an etiological factor for a subset of HNSCC. A vaccine is available for cervical HPV infection, which is recommended for females aged 9 to 26 years and males aged 9 to 21 years (Giuliano *et al.*, 2011). However, oral HPV has been found in 4 to 87% of newborns, and in 52% of children aged 3 to 11 years, suggesting transmission early in life (Martinelli *et al.*, 2012). Therefore, a large percentage of the general population does not benefit from the vaccine. Conversely, HPV is commonly transmitted, and most infections are cleared by the immune system without resulting in pathology. Persistence of HPV infection is the strongest risk factor in the development of cancer (Gillison *et al.*, 2012). Thus, the identification of factors influencing not only the acquisition but also the persistence of HPV infection would lead to more effective prevention and treatment strategies, also benefiting those who are already infected.

HPV has a specific tropism for basal cells of squamous epithelium. The infection begins when the virus gains access through breaks in the mucosa, and the replication of the virus depends on the proliferation of basal cells (Stubenrauch and Laimins, 1999). Mucosal ulcerations and the proliferative state caused by chronic inflammation may facilitate HPV acquisition and persistence within the oral mucosa. The chronically inflamed epithelium is characterized by rete-ridge formation, increasing the surface area exposed. In addition, the candidate HPV receptor, cell-surface heparan sulphate expressed during wound healing, is found extensively in the inflamed epithelium (Hormia

et al., 2005). The results of recent studies have suggested that a history of chronic periodontitis is associated with tumor HPV status in patients with HNSCC. The strength of this association was greater among patients with oropharyngeal compared with those with oral cavity and laryngeal cancers (Tezal *et al.*, 2009b, 2012).

Summary

A model for the role of chronic periodontitis in HNSCC etiology is summarized in Fig. 2. Briefly, chronic periodontitis may be associated with HNSCC by direct toxic effects of bacteria and their products, and/or by indirect effect through inflammation. In addition to its independent effect on carcinogenesis, chronic periodontitis may also facilitate the acquisition and persistence of oral HPV infection.

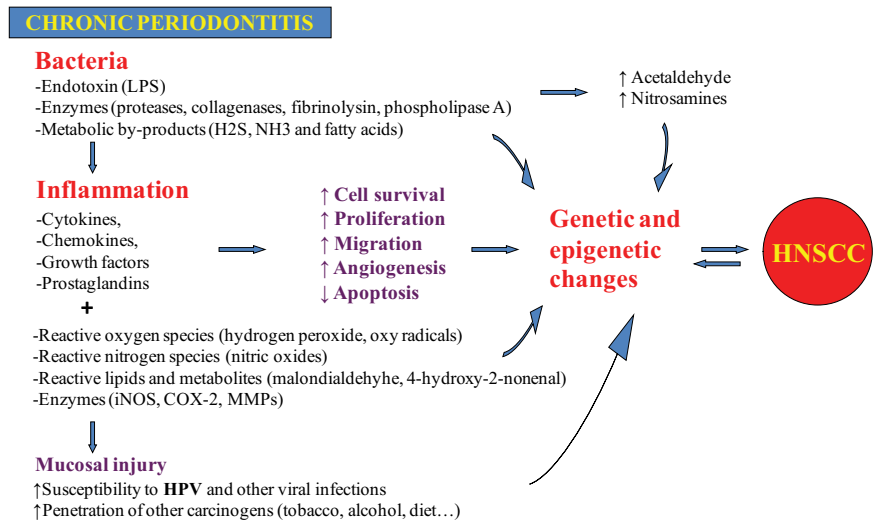

Figure 2. A model for the role of chronic periodontitis in head-and-neck cancer.

CONCLUSIONS

The association between periodontitis and CVD is no longer disputed. Several possible biological mechanisms, including common genetic variants that may explain the link between CVD and periodontitis, have been reviewed. Indeed, it is likely that there are multiple mechanisms underlying these links, with inflammatory, infectious, immune, and genetic components. For example, anti-cardiolipin antibodies are induced by periodontal pathogens in periodontitis patients by molecular mimicry of β2GPI, and these antibodies have biological and pathological activities consistent with those previously reported for other infection-induced antiphospholipid antibodies. These results suggest the hypothesis that anti-Cl may explain some of the observed associations between periodontal infections and systemic conditions such as cardiovascular diseases and adverse pregnancy outcomes. Strategies to inhibit microbial invasion and translocation or to resolve inflammatory responses may be used to treat or prevent pregnancy complications due to bacterial infection.

Associations between periodontal disease and adverse pregnancy outcomes have also been noted. The bacterium *F. nucleatum* has been implicated as a common cause of intra-uterine infection. *F. nucleatum* uses a unique protein to bind to VE-cadherin on endothelial cells and to invade them. These interactions may facilitate translocation of the cadherins to intracellular compartments, thus loosening the cell-cell junction and allowing bacteria in the vicinity to pass through the endothelium (Fardini *et al.*, 2011).

Evidence of an association between chronic periodontitis and HNSCC has practical implications for prevention, early diagnosis, and treatment. Chronic periodontitis may represent a clinical high-risk profile for both oral HPV infection and HNSCC. Periodontal treatment, as an adjunct to conventional oncologic management, may improve the prognosis of HNSCC.

Future research will yield a greater understanding of the relationship between periodontal infection and systemic disease and may enable improved management and prevention strategies to be developed that will benefit both oral and general health.

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