REVIEW ARTICLE

Periodontal Infections and Atherosclerosis: Mechanisms of Association

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ABSTRACT

The bacteria associated with periodontal infection as well the by-products of host-bacterial interactions can have potential systemic effects. The field of periodontal medicine has emerged on the basis of the above and explores the interaction between periodontitis and various systemic conditions, such as cardiovascular disease (CVD), diabetes and pregnancy-related complications. Atherosclerosis constitutes the most important risk factor for serious cardiovascular incidents like myocardial infarction. The links between periodontitis and CVD might be explained by the possible contribution of periodontitis to the formation of atherosclerotic lesions. This review focuses on the mechanism of association between periodontal diseases (PD) and atherosclerosis.

Keywords: Periodontitis, Systemic inflammation, Atherosclerosis.

INTRODUCTION

Periodontitis is an inflammatory condition of the supporting tissues of the teeth with a predominant bacterial etiology. The currently accepted paradigm on the pathogenesis of periodontal disease (PD) is based on the notion that bacterial colonization elicits an immune response by the host that, under additional conditions, may result in the manifestation of clinical disease. It has been well-established that multiple cytokines and inflammatory markers, including IL-1, IL-6, IL-8 and TNF are abundantly produced locally in pathological periodontal tissues. It has been postulated that these locally produced inflammatory mediators are introduced into the bloodstream. Parallel to the induction of short-lived

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bacteremias, chronic periodontal infection has been shown to contribute to a state of systemic inflammation characterized by plasma elevation of acute-phase proteins, such as CRP inflammatory cytokines, such as IL-6 and coagulation factors, such as fibrinogen.

Atherosclerosis has been defined as a progressive disease process that involves the large, to medium-sized muscular and the large elastic arteries. Atherosclerosis can lead to coronary heart disease, as well as myocardial and cerebral infarctions. It is now, widely accepted that a major component of pathology in atherosclerosis involves multiple components of the innate and adaptive immune systems leading to an inflammatory response within the atheromatous lesion.[1] Links between periodontitis and atherosclerosis would be predicted based on inflammatory mechanisms initiated by bacteria associated with periodontal lesions, locally or systemically, that then influence the initiation or propagation of the atherosclerotic lesion.

OVERVIEW OF ATHEROSCLEROSIS PATHOGENESIS

Optimal cardiovascular function requires proper functioning of endothelial cells through the production of paracrine factors that modulate vasodilation, inflammation, thrombosis and cellular proliferation in the blood vessel. Disruption of endothelial function is one of the earliest indicators of cardiovascular disease (CVD), which can be initiated by a number of factors including infection.[2] Following injury, endothelial cells initiate a series of proinflammatory signals, such as the release of chemokines, increased expression of cell adhesion molecules that promote attachment and transmigration of leukocytes into the vascular intima activation of smooth muscle cells and endothelial cell death programs.[3,4]

Damaged endothelia also trigger platelet aggregation and initiate thrombus formation at the site of injury, which can result in vessel occlusion.[5] Activated leukocytes that have migrated into the subendothelial space continue the inflammatory cycle through production of additional proinflammatory cytokines, reactive oxygen species (ROS) and the release of tissue proteinases that degrade the surrounding extracellular matrix. Smooth muscle cells present within the intima and media layer of the vessel also contribute to vascular pathology by secreting matrix metalloproteinases (MMPs) and

undergoing proliferation.[6] Macrophages take up low-density lipoprotein by a process of oxidation (producing oxidized low-density lipoprotein), and are transformed into foam cells in the lamina intima.[7]

MECHANISMS OF ASSOCIATION BETWEEN PERIODONTAL DISEASE AND ATHEROSCLEROSIS

Periodontitis and atherosclerosis appear to have a number of characteristics in common as they are more likely to occur in persons who are older, male, of lower educational status, have fewer financial resources, who smoke, are stressed, and are socially isolated. These commonalties hint that PD and heart disease may share a similar causative pathway. Significantly, periodontal bacteria can affect all of these processes either by directly interacting with/invading endothelial cells, smooth muscle cells, leukocytes and platelets, or indirectly by stimulating the release of paracrine factors that modulate the function of these cells.

DEVELOPMENT OF ENDOTHELIAL DYSFUNCTION AND FORMATION OF ATHEROMATOUS PLAQUE BY PERIODONTAL PATHOGENS AND THEIR PRODUCTS

Entry of oral bacteria and/or bacterial products into the bloodstream is thought to be one of the key initiators of biological events that link oral infections to atherosclerosis. Bacterial DNA from several periodontal pathogens has been detected in human endarterectomy specimens.[8] Viable *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* were recovered and cultured from human atheromatous plaques originating from a patient with PD.[9]

In addition to whole bacteria-endothelial cell interactions, studies have examined the effects of specific bacterial products, such as microbial proteases, lipopolysaccharide (LPS) and outer membrane vesicles (OMVs). Arginine-specific gingipain, a *P. gingivalis*-specific protease increased the responsiveness of endothelial cells to live *P. gingivalis* and *P. gingivalis* LPS, by inducing Weibel-Palade body exocytosis through activation of protease-activated receptors (PARs). Weibel-Palade bodies are vesicles in endothelial cells that store vasoactive substances, such as angiopoietin-2, which may enhance IL-8 production by LPS-stimulated cells.[10]

Outer membrane vesicles are vesicles 'budding off' from growing bacterial cells and comprising a protein fraction and LPS. *Porphyromonas gingivalis* OMVs were found to impair growth and tube formation endothelial cells, an effect mediated by the protein fraction of OMVs.[11] In addition, a free-soluble surface material, released by *A. actinomycetemcomitans* grown either in a biofilm or in a planktonic form, was found to induce production of several proinflammatory cytokines in human whole blood. Since both OMVs and free soluble surface material are abundantly produced locally in the plaque biofilm, their potential entry into the circulation may constitute a significant source of inflammatory stimulants along with the planktonic bacteria in the bloodstream.

Activation of Vascular Endothelium

Upon entering the bloodstream, bacteria are rapidly cleared by host immune cells. To survive and elicit effects at distant sites, they have evolved several host-evasion strategies like invasion of vascular endothelial cells by these microorganisms. Within the endothelial cell, the survival of *P. gingivalis* depends on the concurrent activation of autophagy and suppression of apoptosis, which provides an intracellular niche where the pathogen can replicate unobstructed by host immune responses.

Porphyromonas gingivalis invasion of endothelial cells is dependent on fimbriae and a specific hemagglutinin. Fimbriae appear to be critical for both the invasive and proatherogenic properties of *P. gingivalis*. Induction of IL-6 in vascular endothelial cells has also been shown to be a process dependent on fimbriae, nuclear factor-kappa B (NF-κB), and meiosis-specific kinase 1, which is regulated by the autocrine IL-6 signal transducer gp130.[12] Expression of the chemokine monocyte chemoattractant protein-1 (MCP-1), an important regulator of monocyte migration from the vessel lumen to the subendothelial space, was strongly induced by *P. gingivalis* infection.

Induction of Apoptosis in Endothelium by Periodontal Pathogens

Induction of apoptosis in vascular endothelial cells, a hallmark of developing endothelial dysfunction is another bacterial strategy of critical importance in atherogenesis. *Porphyromonas gingivalis* gingipains were shown to induce cell adhesion molecule cleavage, detachment, and apoptotic cell death in bovine coronary artery endothelial cells.[13] Both gingipains and whole *P. gingivalis* were also able to induce caspase-independent programed cell death.[14,15]

Contributions of Periodontal Pathogens to the Formation of Fatty Streaks and Atherosclerotic Plaques

Cell-free products of *Porphyromonas gingivalis* were shown to induce proliferation of aortic smooth-muscle cells *in vitro* after preincubation with human plasma.[16] *Porphyromonas gingivalis*-mediated proliferation in human endothelial cells, including tube formation, and angiogenesis in matrigel plugs was found to be dependent on activation of the mitogen-activated extracellular signal-regulated kinase-1 and-2 (ERK1/2).[17]

Dyslipidemic Effect by Periodontal Pathogens

It has been postulated that cholesterol biosynthesis and transport are influenced by infectious processes. Pertinent to periodontitis, the presence of LPS in plasma and acute-phase responses to systemic dissemination of bacteria could promote elevated biosynthesis of cholesterol in the liver, which in turn is transported as serum lipids capable of binding to bacterial LPS. In this manner, a pathway can be envisioned in which periodontal infection both promotes dyslipidemia and interacts with serum lipids so as to enhance their atherogenicity.[18]

Interactions with Monocytes/Tissue Macrophages and Enhanced LDL-uptake and Foam Cell Formation

There is an increased adhesion of monocytes to human aortic endothelial cells infected with invasive *P. gingivalis* which is mediated by elevated expression of adhesion molecules and chemotactic cytokines in the endothelial cells.[19] Infection of monocytes with invasive strains of *P. gingivalis* enhanced migration and elicited the expression of the proinflammatory cytokines TNF-alpha and IL-6.[20]

Monocyte infection with invasive *P. gingivalis* strains also promote enhanced LDL-uptake and foam cell formation.[21] The presence of whole bacterial cells is not necessary for these effects. Indeed, LPS-challenged monocytes derived macrophages showed enhanced secretion of TNF-alpha and interleukin-1 beta and induction of foam cell formation and accumulation of LDL. Lipopolysaccharide stimulation also decreased mRNA levels of scavenger receptor B, and ATP-binding cassette transporter-1, i.e. of two receptors that mediate the efflux of cholesterol from macrophages.[22,23]

Role of Periodontal Pathogens in Enhanced Prothrombotic State

The pathogens or their products can activate the platelets directly or indirectly via the vascular endothelium. Platelet aggregation in plasma was shown to depend on the adhesion molecule Hgp44 and the *P. gingivalis* protease Lys-gingipain (Kgp).[24] *Porphyromonas gingivalis* had a sensitizing effect on human platelets, enhancing epinephrine-induced aggregation. This effect was attributed to a limited activation of PARs on the platelet surface by gingipains, with subsequent mobilization of Ca^{2+}, leading to a marked coagulant response to epinephrine binding to the alpha-2 adrenergic receptor.[25]

Porphyromonas gingivalis gingipains induce hydrolysis of platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31), which enhance the vascular permeability.[26] Infection of endothelial cells with invasive

P. gingivalis strains resulted in enhanced tissue factor expression and activity, suppressed levels of tissue factor inhibitor, decreased levels and activity of tissue plasminogen activator, and increased plasminogen activator inhibitor-1 antigen levels.[19] *Porphyromonas gingivalis* arginine- and lysine-specific gingipains induce degradation of vascular endothelial cell thrombomodulin.[11] Elevated levels of platelet-activating factor (PAF) and P-selectin, a marker of platelet activation, were documented in the plasma of patients with periodontitis and atherosclerosis.[27] Furthermore, platelets from periodontitis patients showed an increased binding of the glycoprotein IIb-IIIa complex, a direct measure of platelet activation, which correlated positively with the extent and severity of periodontitis of the donor. A higher platelet expression of P-selectin, and increased formation of platelet-monocyte complexes in periodontitis patients were also demonstrated.[28] The platelet/monocyte complexes displayed a better ability to bind and phagocytose periodontal pathogens suggesting that increased atherothrombosis was paralleled by enhanced bacterial clearance.

Elevated fibrinogen is an indicator of systemic inflammation and results in increased blood viscosity. Fibrinogen and its degradation products can be localized to atheromas as a structural component of the lesion where it, and its degradation products, can induce inflammatory cytokine production as well as promote platelet aggregation.[29] Patients with periodontitis have higher plasma fibrinogen levels and white blood cell counts than age matched controls, and suggested a link to CVD.

In addition to mechanism by platelet activation, prothrombotic effect can also occur by means of pathogen-mediated apoptosis of vascular endothelial cells. Activated endothelium by either direct interaction with periodontal pathogens, or via systemic inflammatory molecules, is known to express tissue factor (thrombokinase), an important mediator of thrombin formation. However, tissue factor expressed on vascular endothelium is often encrypted, i.e. rendered ineffective by post-translational modification and, thus, is unable to exert its procoagulant function. Apoptosis of endothelial cells can decrypt tissue factor by increasing calcium concentrations and proteolytic cleavage and, thereby can trigger thrombosis, even when the basement membrane is not uncovered by endothelial desquamation.[30]

Role of Periodontal Pathogens in Rupture of Atheromatous Plaques

Degradation of fibrous caps is mediated by MMPs produced within the atheromatous plaques by macrophages. Periodontal pathogens, like *P. gingivalis* and *Prevotella intermedia* have been reported to induce production of

several MMPs in different cell types, including macrophages and endothelial cells, and reduce the expression of the MMP antagonist tissue inhibitor of MMPs (TIMPs). *Porphyromonas gingivalis* was shown to degrade fibrous cap material isolated from human autopsy plaque samples *in vitro*.[31] The proapoptotic effects of periodontal pathogens can also contribute to plaque erosion. The reported proinflammatory effects of *A. actinomycetemcomitans* in human mast cells may also be relevant in this context.[32] Although mast cells are uncommon in vascular tissues, they do localize in atherosclerotic plaques and particularly in shoulder regions of rupture prone plaques.[33] Activation of this cell population and subsequent production of levels of proteases capable of destabilization of atheromatic plaques correlate with intraplaque hemorrhage, endothelial cell and macrophage apoptosis and vascular leakage.[34]

ACTIVATION OF INNATE IMMUNE SIGNALLING AND AUTOIMMUNE RESPONSES TO PERIODONTAL PATHOGENS

Toll-like receptors (TLRs) and other pattern recognition receptors (PRRs) are involved in exerting proatherogenic effects in vascular endothelial cells. These primary receptors of the innate immune system recognize highly conserved pathogen-associated molecular patterns (PAMPs). Activation of TLRs and their downstream signaling pathways leads to cellular activation and a specific response to microbial infection. Expression of TLRs is strongly induced in endothelial cells and macrophages in atherosclerotic lesions. *Porphyromonas gingivalis* LPS appears to interact with different TLRs in a cell-type dependent manner.[35] The activation of human vascular endothelial cells, monocytes, and macrophages by *P. gingivalis* was mediated by TLR2, but not by TLR4. Hypercholesterolemia may result in accelerated TLR2-mediated atherosclerosis.[36]

Autoimmune Responses to Periodontal Bacteria-Molecular Mimicry

Periodontitis patients known to have elevated systemic antibody responses to microorganisms, and several such organisms are known to be able to induce cross-reactive and specific antibodies of relevance to atherosclerosis risk. Measures of such antibodies have both been associated with increased cardiovascular risk in periodontitis. Molecular mimicry occur when sequence similarities between foreign and self-peptides produce cross-activation of autoreactive T or B cells that can lead to tissue pathology or autoimmunity.

A family of highly conserved heat-shock proteins (HSPs) may be expressed on certain bacterial membranes,

when exposed to stress. Bacterial HSPs are considered major antigenic determinants that elicit antibodies and specific reactive T-cells that can cross-react with host cells expressing homologous molecules, resulting in auto-aggressive destruction. High degree of homology was found between the *P. gingivalis* HSP60—termed GroEL—and mammalian HSP60 family members.[37] *Porphyromonas gingivalis* GroEL was shown to be highly immunogenic, and was recognized by serum antibodies isolated from patients suffering from PD.[38]

Another family of antibodies namely anticardiolipin (anti-CL) antibodies, are known to be associated with vascular thrombosis and early atherosclerosis. It has been shown that a variety of microbial pathogens are capable of inducing pathogenic anti-CL because of their similarities to peptide sequences in target antigen site. Patients with chronic or aggressive periodontitis demonstrate a higher prevalence of elevated levels of anti-CL than healthy subjects without periodontitis. An association between levels of serum anti-CL and serum markers of vascular inflammation, including sICAM-1, sVCAM-1, and sE-selectin, were found in periodontitis patients.[39] Anti-phosphorylcholine (anti-PC) and antioxidized LDL (anti-oxLDL) are other antibodies implicated in risk for CVD. They have been also shown to be inducible by periodontal pathogens.[40]

INDUCTION OF OXIDATIVE STRESS BY PERIODONTAL PATHOGENS

Induction of oxidative stress is another potential pathway through which periodontitis may contribute to atherogenesis. Oxidation of LDL via ROS is a prerequisite for cholesterol uptake by macrophages and the formation of foam cells but also results in several additional proatherogenic effects.[41,42] Thus, apart from its involvement in the formation of fatty streaks, Ox-LDL also affects the vascular endothelium both directly and indirectly. Direct effects include the induction of cellular activation and apoptosis by interaction with lectin-like oxidized low-density lipoprotein receptor (LOX-1).[43] Indirect effects are exerted through downregulation of the expression of endothelial nitric oxide synthase (eNOS), which results in increased production of ROS, ongoing LDL oxidation, and endothelial dysfunction.[44] Ox-LDL also inhibits differentiation and induces apoptosis of endothelial progenitor cells (EPCs), a subpopulation of bone marrow-derived stem cells that participates in vascular repair.[45] It has been established that Ox-LDL up-regulates proatherogenic chemokines and adhesion molecules via the CD40/CD40L pathway and triggers IL-6, TNF-alpha and CRP secretion.[46]

CONFOUNDING ROLE OF SHARED RISK FACTORS

Periodontal disease and atherosclerosis share many risk factors, such as increasing age, smoking, alcohol abuse, race/ethnicity, education and socioeconomic status, male sex, diabetes mellitus, and overweight or obesity. The impact of these factors may confound the relationship between two conditions. Smoking is a major risk factor for both periodontal and CVD. The role of smoking in the observed association between PD and CVD outcomes is a critical one because smoking can play a role both as a confounder and as an effect modifier.

Systemic inflammation, defined by increased circulating TNF-α, is associated with obesity and periodontitis and has been proposed as a mechanism for the connection between these conditions.[47] Systemic inflammatory responses also could explain the association between periodontitis and type 2 diabetes by cytokine induced insulin resistance.[47] Increased hsCRP plasma levels in patients with prehypertension and patients with established hypertension, may link two conditions.[48] Major depression, physical inactivity, family histories of CVD and PD, advancing age, and male gender are other risk factors for atherosclerotic CVD that are commonly found in patients with periodontitis and also may serve as confounders.[49]

CONCLUSION

The relationship between PD and atherosclerosis is potentially of great public health importance because of their high prevalence. It seems clear that several mechanistic pathways may exist that explain how periodontitis might be causally linked to atherosclerosis. These biological explanations most likely occur simultaneously and could be the direct or indirect consequence of the pathogenic microbiota in periodontal lesions. However, a direct causal relationship between periodontitis and atherosclerosis has not been established. Exploration of any such mechanism by future studies may ultimately aid in reducing the morbidity and mortality associated with CVD by a combined treatment approach by dental and medical specialists.

REFERENCES

1. Libby P, Ridker PM, Hansson GK. Inflammation in atherosclerosis: from pathophysiology to practice. J Am Coll Cardiol 2009;54(23):2129-2138.
2. Vita JA, Loscalzo J. Shouldering the risk factor burden: infection, atherosclerosis, and the vascular endothelium. Circulation 2002;106(2):164-166.
3. Woollard KJ, Geissmann F. Monocytes in atherosclerosis: subsets and functions. Nat Rev Cardiol 2010;7(2):77-86.
4. Goveia J, Stapor P, Carmeliet P. Principles of targeting endothelial cell metabolism to treat angiogenesis and endothelial cell dysfunction in disease. EMBO Mol Med 2014;6(9):1105-1120.
5. Popovic M, Smiljanic K, Dobutovic B, Syrovets T, Simmet T, Isenovic ER. Human cytomegalovirus infection and atherothrombosis. J Thrombo Thrombolysis 2012;33(2):160-172.
6. Prochnau D, Lehmann M, Straube E, Figulla HR, Rodel J. Human cytomegalovirus induces MMP-1 and MMP-3 expression in aortic smooth muscle cells. Acta Microbiologica et Immunologica Hungarica 2011;58(4):303-317.
7. Glass CK, Witzum JL. Atherosclerosis: the road ahead. Cell 2001;104(4):503-516.
8. Zaremba M, Gorska R, Suwalski P, Kowalski J. Evaluation of the incidence of periodontitis-associated bacteria in the atherosclerotic plaque of coronary blood vessels. J Periodontol 2007;78(2):322-327.
9. Kozarov EV, Dorn BR, Shelburne CE, Dunn WA Jr, Progulske-Fox A. Human atherosclerotic plaque contains viable invasive Actinobacillus actinomycetemcomitans and porphyromonas gingivalis. Arterioscler Thromb Vasc Biol 2005;25(3):e17-e18.
10. Bartruff JB, Yukna RA, Layman DL. Outer membrane vesicles from porphyromonas gingivalis affect the growth and function of cultured human gingival fibroblasts and umbilical vein endothelial cells. J Periodontol 2005;76(6):972-979.
11. Ho Y-S, Lai M-T, Liu S-J, Lin C-T, Naruishi K, Takashiba S, et al. Porphyromonas gingivalis fimbriae-dependent interleukin-6 autocrine regulation by increase of gp130 in endothelial cells. J Periodontal Res 2009;44(4):550-556.
12. Inomata M, Into T, Ishihara Y, Nakashima M, Noguchi T, Matsushita K. Arginine-specific gingipain A from Porphyromonas gingivalis induces Weibel-Palade body exocytosis and enhanced activation of vascular endothelial cells through protease-activated receptors. Microbes Infect 2007;9(12-13):1500-1506.
13. Sheets SM, Potempa J, Travis J, Casiano CA, Fletcher HM. Gingipains from porphyromonas gingivalis W83 induce cell adhesion molecule cleavage and apoptosis in endothelial cells. Infect Immun 2005;73(3):1543-1552.
14. Desta T, Graves DT. Fibroblast apoptosis induced by Porphyromonas gingivalis is stimulated by a gingipain and caspase-independent pathway that involves apoptosis-inducing factor. Cell Microbiol 2005;9(11):2667-2675.
15. Roth GA, Ankersmit HJ, Brown VB, Papapanou PN, Schmidt AM, Lalla E. Porphyromonas gingivalis infection and cell death in human aortic endothelial cells. FEMS Microbiol Lett 2007;272(1):106-113.
16. Inaba H, Hokamura K, Nakano K, Nomura R, Katayama K, Nakajima A, et al. Upregulation of S100 calcium-binding protein A9 is required for induction of smooth muscle cell proliferation by a periodontal pathogen. FEBS Lett 2009;583(1):128-134.
17. Koo TH, Jun HO, Bae S-K, Kim S-R, Moon C-P, Jeong S-K, et al. Porphyromonas gingivalis, periodontal pathogen, lipopolysaccharide induces angiogenesis via extracellular signal-regulated kinase 1/2 activation in human vascular endothelial cells. Arch Pharm Res 2007;30(1):34-42.
18. Schenkein HA, Loos BG. Inflammatory mechanisms linking periodontal diseases to cardiovascular diseases. J Periodontol 2013;84(Suppl 4):S51-S69.
19. Roth GA, Moser B, Roth-Walter F, Giacona MB, Harja E, Papapanou PN, et al. Infection with a periodontal pathogen

increases mononuclear cell adhesion to human aortic endothelial cells. Atherosclerosis 2007;190(2):271-281.

20. Pollreisz A, Huang Y, Roth G, Cheng B, Kebschull M, Papapanou PN, et al. Enhanced monocyte migration and proinflammatory cytokine production by Porphyromonas gingivalis infection. J Periodontal Res 2010;45(2):239-245.

21. Giacona MB, Papapanou PN, Lamster IB, Rong LL, D'Agati VD, Schmidt AM, et al. Porphyromonas gingivalis induces its uptake by human macrophages and promotes foam cell formation in vitro. FEMS Microbiol Lett 2004;241(1): 95-101.

22. Lakio L, Lehto M, Tuomainen AM, Jauhiainen M, Malle E, Asikainen S, et al. Proatherogenic properties of lipopolysaccharide from the periodontal pathogen Actinobacillus actinomycetemcomitans. J Endotoxin Res 2006;12(1):57-64.

23. McGillicuddy FC, de la Llera Moya M, Hinkle CC, Joshi MR, Chiquoine EH, Billheimer JT, et al. Inflammation impairs reverse cholesterol transport in vivo. Circulation 2009;119(8): 1135-1145.

24. Naito M, Sakai E, Shi Y, Ideguchi H, Shoji M, Ohara N, et al. Porphyromonas gingivalis-induced platelet aggregation in plasma depends on Hgp44 adhesin but not Rgp proteinase. Mol Microbiol 2006;59(1):152-167.

25. Nylander M, Lindahl TL, Bengtsson T, Grenegård M. The periodontal pathogen Porphyromonas gingivalis sensitises human blood platelets to epinephrine. Platelets 2008;19(5): 352-358.

26. Yun PLW, Decarlo AA, Chapple CC, Hunter N. Functional implication of the hydrolysis of platelet endothelial cell adhesion molecule 1 (CD31) by gingipains of Porphyromonas gingivalis for the pathology of periodontal disease. Infect Immun 2005;73(3):1386-1398.

27. Papapanagiotou D, Nicu EA, Bizzarro S, Gerdes VE, Meijers JC, Nieuwland R, et al. Periodontitis is associated with platelet activation. Atherosclerosis 2009;202(2):605-611.

28. Nicu EA, Van der Velden U, Nieuwland R, Everts V, Loos BG. Elevated platelet and leukocyte response to oral bacteria in periodontitis. J Thromb Haemost 2009;7(1):162-170.

29. Davalos D, Akassoglou K. Fibrinogen as a key regulator of inflammation in disease. Semin Immunopathol 2012;34(1): 43-62.

30. Greeno EW, Bach RR, Moldow CF. Apoptosis is associated with increased cell surface tissue factor procoagulant activity. Lab Invest 1996;75(2):281-289.

31. Kuramitsu HK, Qi M, Kang IC, Chen W. Role for periodontal bacteria in cardiovascular diseases. Ann Periodontol 2001;6(1):41-47.

32. Oksaharju A, Lappalainen J, Tuomainen AM, Pussinen PJ, Puolakkainen M, Kovanen PT, et al. Proatherogenic lung and oral pathogens induce an inflammatory response in human and mouse mast cells. J Cell Mol Med 2009;13(1):103-113.

33. Lindstedt KA, Mayranpaa MI, Kovanen PT. Mast cells in vulnerable atherosclerotic plaques—a view to a kill. J Cell Mol Med 2007;11(4):739-758.

34. Bot I, de Jager SC, Zernecke A, Lindstedt KA, van Berkel TJ, Weber C, et al. Perivascular mast cells promote atherogenesis and induce plaque destabilization in apolipoprotein E-deficient mice. Circulation 2007;115(19):2516-2525.

35. Kocgozlu L, Elkaim R, Tenenbaum H, Werner S. Variable cell responses to P gingivalis lipopolysaccharide. J Dent Res 2009;88(8):741-745.

36. Wang M, Hajishengallis G. Lipid raft-dependent uptake, signalling and intracellular fate of porphyromonas gingivalis in mouse macrophages. Cell Microbiol 2008;10(10):2029-2042.

37. Maeda H, Miyamoto M, Hongyo H, Nagai A, Kurihara H, Murayama Y. Heat shock protein 60 (GroEL) from porphyromonas gingivalis: molecular cloning and sequence analysis of its gene and purification of the recombinant protein. FEMS Microbiol Lett 1994;119(1-2):129-135.

38. Ford PJ, Gemmell E, Chan A, Carter CL, Walker PJ, Bird PS, et al. Inflammation, heat shock proteins and periodontal pathogens in atherosclerosis: an immunohistologic study. Oral Microbiol Immunol 2006;21(4):206-211.

39. Schenkein HA, Best AM, Brooks CN, Burmeister JA, Arrowood JA, Kontos MC, Tew JG. Anticardiolipin and increased serum adhesion molecule levels in patients with aggressive periodontitis. J Periodontol 2007;78(3):459-466.

40. Schenkein HA, Berry CR, Burmeister JA, Brooks CN, Best AM, Tew JG. Locally produced anti-phosphorylcholine and antioxidized low-density lipoprotein antibodies in gingival crevicular fluid from aggressive periodontitis patients. J Periodontol 2004;75(1):146-153.

41. Itabe H. Oxidative modification of LDL: its pathological role in atherosclerosis. Clin Rev Allergy Immunol 2009;37(1):4-11.

42. Verhoye E, Langlois MR. Circulating oxidized low-density lipoprotein: a biomarker of atherosclerosis and cardiovascular risk? Clin Chem Lab Med 2009;47(2):128-137.

43. Ma FX, Zhou B, Chen Z, Ren Q, Lu SH, Sawamura T, et al. Oxidized low density lipoprotein impairs endothelial progenitor cells by regulation of endothelial nitric oxide synthase. J Lipid Res 2006;47(6):1227-1237.

44. Victor VM, Rocha M, Sola E, Banuls C, Garcia-Malpartida K, Hernandez-Mijares A. Oxidative stress, endothelial dysfunction and atherosclerosis. Curr Pharm Des 2009;15(26): 2988-3002.

45. Zenovich AG, Taylor DA. Atherosclerosis as a disease of failed endogenous repair. Front Biosci 2008 May;13:3621-3636.

46. Hulthe J, Fagerberg B. Circulating oxidized LDL is associated with subclinical atherosclerosis development and inflammatory cytokines (AIR Study). Arterioscler Thromb Vasc Biol 2002;22(7):1162-1167.

47. Genco RJ, Grossi SG, Ho A, Nishimura F, Murayama Y. A proposed model linking inflammation to obesity, diabetes, and periodontal infections. J Periodontol 2005;76(Suppl 11):2075-2084.

48. Sesso HD, Buring JE, Rafai N, Blake GJ, Gaziano JM, Ridker PM. C-reactive protein and the risk of developing hypertension. JAMA 2003;290(22):2945-2951.

49. Friedewald VE, Kornman KS, et al. The American Journal of Cardiology and Journal of Periodontology Editors' Consensus: Periodontitis and Atherosclerotic Cardiovascular Disease. J Periodontol 2009;80(7):1021-1032.