

Pathogen-induced inflammation at sites distant from oral infection: bacterial persistence and induction of cell-specific innate immune inflammatory pathways

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SUMMARY

A hallmark of infection with the gram-negative pathogen *Porphyromonas gingivalis* is the induction of a chronic inflammatory response. *P. gingivalis* induces a local chronic inflammatory response that results in oral inflammatory bone destruction, which manifests as periodontal disease. In addition to chronic inflammation at the initial site of infection, mounting evidence has accumulated supporting a role for *P. gingivalis*-mediated periodontal disease as a risk factor for several systemic diseases including, diabetes, preterm birth, stroke, and atherosclerotic cardiovascular disease. A growing number of *in vitro* studies have demonstrated that *P. gingivalis* infection stimulates cell activation commensurate with expected responses paralleling inflammatory atherosclerotic-type responses. Furthermore, various mouse models have been used to examine the ability of *P. gingivalis* to stimulate chronic inflammatory plaque accumulation and recent studies have pointed to a pivotal role for innate immune signaling via the Toll-like receptors in the chronic inflammation associated with *P. gingivalis* infection. In this review we discuss the pathogen and host cell specificity of these responses and discuss possible mechanisms by which this oral pathogen can induce and main-

tain a chronic state of inflammation at sites distant from oral infection.

INTRODUCTION

Porphyromonas gingivalis is a primary etiological agent of human periodontal disease, an inflammatory disease that affects approximately 100 million people in the USA. *P. gingivalis* induces a local chronic host inflammatory response that results in inflammatory bone destruction, which is manifested as periodontal disease (Oliver *et al.*, 1998). In humans, the local chronic inflammatory lesions associated with *P. gingivalis* infection are initiated at the vascular endothelium in the oral cavity (Pihlstrom *et al.*, 2005). During acute or active stages of periodontal disease, there is an initial neutrophilic cellular infiltrate that switches to a predominating monocytic and lymphocytic cellular infiltrate in chronic lesions. Macrophages in inflamed tissues differentiate to osteoclasts and also accelerate bone resorption through the production of proinflammatory cytokines (Adamopoulos *et al.*, 2006). A number of proinflammatory mediators are expressed in periodontal inflammatory lesions and many of these are also expressed systemically (D'Aiuto *et al.*, 2004).

It has been postulated that the ability to control the expression of these proinflammatory mediators is crucial to controlling the bone loss and subsequent pathology associated with *P. gingivalis* infection.

SYSTEMIC COMPLICATIONS OF *P. GINGIVALIS*-INDUCED CHRONIC INFLAMMATION

Mounting evidence has accumulated supporting a role for *P. gingivalis*-mediated periodontal disease as a risk factor for several systemic diseases including atherosclerotic cardiovascular disease (Genco, 1996; Morrison *et al.*, 1999; Genco *et al.*, 2002). Although not all reports conclude that there is an association between *P. gingivalis*-mediated periodontal disease and atherosclerosis, recent results from the Oral Infections and Vascular Disease Epidemiology Study revealed that there was an association between periodontal pathogens and subclinical atherosclerosis (Desvarieux *et al.*, 2005). Clinical identifiers of periodontal disease including increased probing depth, bleeding on probing, clinical attachment loss, tooth number, and radiographic assessments have been linked with endothelial dysfunction (Amar *et al.*, 2003), aortic valve stenosis (Volzke *et al.*, 2005), and early carotid atherosclerosis (Engebretson *et al.*, 2005; Soder *et al.*, 2005). Furthermore, periodontal treatment studies have been associated with improved endothelial dysfunction (Seinost *et al.*, 2005). In addition to clinical studies, further evidence of linkage comes from reports that *P. gingivalis*-specific DNA is present in inflammatory atherosclerotic plaques (Padilla *et al.*, 2006).

ANIMAL MODELS OF *P. GINGIVALIS*-INDUCED ATHEROSCLEROSIS

Inflammation plays a key role in the development/progression of atherosclerosis (Libby, 2000; Gibson *et al.*, 2008) and various animal models have been used to examine the ability of *P. gingivalis* to stimulate an inflammatory response associated with atherosclerosis. Studies in rabbit and pig models have demonstrated that *P. gingivalis* challenge accelerates vascular intimal thickening and accelerates atherosclerosis (Jain *et al.*, 2003; Brodala *et al.*, 2005). In a murine *apolipoprotein E*-deficient (ApoE^{-/-}) hyperlipidemic mouse model, repeated injection of *P. gingivalis* into the tail vein was demonstrated to accelerate

atherosclerosis (Li *et al.*, 2002). In a more biologically relevant ApoE^{-/-} mouse model, we reported that *P. gingivalis* oral infection accelerates inflammatory atherosclerosis (Gibson *et al.*, 2004). We also demonstrated that the *P. gingivalis*-accelerated atherosclerosis occurred early after infection and was prevented by immunization before challenge (Gibson *et al.*, 2004; Miyamoto *et al.*, 2006).

Adherence to and invasion of vascular tissue by *P. gingivalis* likely plays a key role in the processes that lead to stimulation of accelerated atheroma development, as oral challenge of ApoE^{-/-} mice with a *P. gingivalis* *fimA* mutant failed to accelerate atherosclerosis (Gibson *et al.*, 2004; Miyamoto *et al.*, 2006). Mice infected with invasive *P. gingivalis* expressed increased levels of macrophages in the inflammatory lesions and increased expression of cell-associated intercellular adhesion molecule-1, vascular cell adhesion molecule-1, Toll-like receptor 2 (TLR2), and TLR4 early following infection (Gibson *et al.*, 2004; Miyamoto *et al.*, 2006). Although our data support the hypothesis that both the wild-type and the *fimA* mutant can gain access into the vasculature and to the aorta, the *fimA* mutant failed to elicit the induction of inflammatory molecules by the endothelium, and resembled unchallenged controls (Gibson *et al.*, 2004).

TLR-MEDIATED INFLAMMATORY PATHWAYS AND *P. GINGIVALIS* INFLAMMATION *IN VIVO*

Activation of proinflammatory cytokines occurs in part via the TLRs, a family of innate immune recognition receptors that detect conserved microbial patterns and endogenous ligands and play a key role in innate immune signaling and initiating inflammatory responses (Akira *et al.*, 2006). Ligation of these receptors initiates the activation of several transcription factors including nuclear factor- κ B, resulting in the expression of a wide array of inflammatory genes. Recent studies have established that in humans, polymorphisms in genes encoding TLRs and genes involved in subsequent signaling cascades are associated with atherosclerosis (Kiechl *et al.*, 2002; Bjorkbacka, 2006; Hansson & Libby, 2006; Pryshchep *et al.*, 2008). Animal studies employing hyperlipidemic mice have shown that TLR2, TLR4, and the downstream signaling molecules myeloid differentiation factor (MyD88) and interleukin-1 receptor-associated kinase 4 play an important role in the

progression of atherosclerosis (Bjorkbacka *et al.*, 2004; Michelsen *et al.*, 2004; Mullick *et al.*, 2005; Liu *et al.*, 2008; Rekhter *et al.*, 2008). Using an ApoE^{-/-} mouse model we recently demonstrated that TLR2 contributes to the induction of proinflammatory responses in atherosclerotic lesions in response to infection with *P. gingivalis* (Hayashi *et al.*, 2010). *P. gingivalis* infection in mice possessing functional TLR2 induced the accumulation of macrophages, and inflammatory mediators including CD40, interferon- γ and the proinflammatory cytokines interleukin-1 β (IL-1 β), IL-6 and tumor necrosis factor- α (TNF- α) in atherosclerotic lesions. The expression of these inflammatory mediators was reduced in atherosclerotic lesions from *P. gingivalis*-infected TLR2-deficient (TLR2^{-/-}) mice. Interestingly we also observed that *P. gingivalis* infection resulted in greater changes in the levels of inflammatory mediators present in atherosclerotic plaque samples compared with the levels of inflammatory mediators observed in serum at the time of sacrifice. These results suggest that while *P. gingivalis*-induced systemic inflammation may contribute to atherosclerosis, the ability to modulate the levels of inflammatory mediators present in atherosclerotic plaques may ultimately be responsible for the induction of atherosclerosis. We also observed that while the absence of TLR2 diminished atherosclerosis in response to *P. gingivalis* infection, a TLR2-independent mechanism may also play a role in *P. gingivalis*-mediated inflammatory responses in atherosclerosis.

PATHOGEN SPECIFICITY IN INDUCTION OF CHRONIC INFLAMMATION AND ATHEROSCLEROSIS

We recently performed studies to determine if chronic bacterial infection and systemic inflammation are sufficient to drive atherosclerosis. In these studies we orally challenged groups of ApoE^{-/-} mice with *Helicobacter pylori*, an organism that causes gastritis and peptic ulcers (Kusters *et al.*, 2006) or *P. gingivalis*. Gastritis and oral bone loss were observed only in the groups of mice challenged with *H. pylori* or *P. gingivalis*, respectively (Fig. 1A–C and data not shown). Mice challenged with *P. gingivalis* exhibited increases in the distance between the alveolar bone crest to the cementum–enamel junction, relative to uninfected mice ($P < 0.05$), whereas in mice orally challenged with *H. pylori* the oral bone measure-

ments resembled those of the control group (data not shown). Histological evaluation of stomach tissue cryosections revealed that ApoE^{-/-} mice challenged with *H. pylori* presented with a characteristic submucosal cellular infiltrate (Fig. 1B). Viable *H. pylori* were isolated from stomach tissues (data not shown) confirming bacterial colonization. As expected, we did not observe a cellular infiltrate in gastric tissue samples obtained from *P. gingivalis*-infected ApoE^{-/-} mice, and these tissues resembled the unchallenged, age-matched controls (Fig. 1C). Serum analysis revealed elevated levels of *H. pylori*-specific and *P. gingivalis*-specific immunoglobulin G only in the groups of mice challenged with the respective pathogen (data not shown). Morphometric analysis of digital micrographs following Sudan IV staining revealed that ApoE^{-/-} mice challenged with *P. gingivalis* possessed significantly more atherosclerotic plaque in the aortic arch than either the *H. pylori*-infected or unchallenged groups of animals ($P < 0.05$ and $P < 0.05$, respectively; Fig. 1D–G). In contrast, Sudan IV staining of the aorta of *H. pylori*-infected ApoE^{-/-} mice revealed similarity with the unchallenged control group. These results indicate that there is bacterial specificity in pathogen-induced chronic infection and acceleration of atherosclerosis. Analysis of sera from mice challenged with *H. pylori* and *P. gingivalis* revealed that only the ApoE^{-/-} mice challenged with *P. gingivalis* expressed elevated serum levels of the proinflammatory cytokine IL-1 β compared with unchallenged or *H. pylori*-challenged mice (Fig. 1H). Other studies have reported that the IL-1 pathway plays an important role in pathogen-accelerated atherosclerosis. Using mice that were heterozygous at the *apoE* allele (ApoE^{+/-}) placed on high-fat diet and deficient in IL-1 receptor it was reported that *P. gingivalis* challenge did not accelerate atherosclerotic plaque (Chi *et al.*, 2004). Despite several differences in assay conditions between our present study and the study of Chi *et al.*, including diet, ApoE, and IL-1 receptor alleles, collectively these studies support a role for the IL-1 pathway in *P. gingivalis*-accelerated atherosclerosis.

P. GINGIVALIS INDUCED INFLAMMATORY SIGNALING CASCADES

Both *P. gingivalis* and defined antigens of this organism induce a potent inflammatory response in various

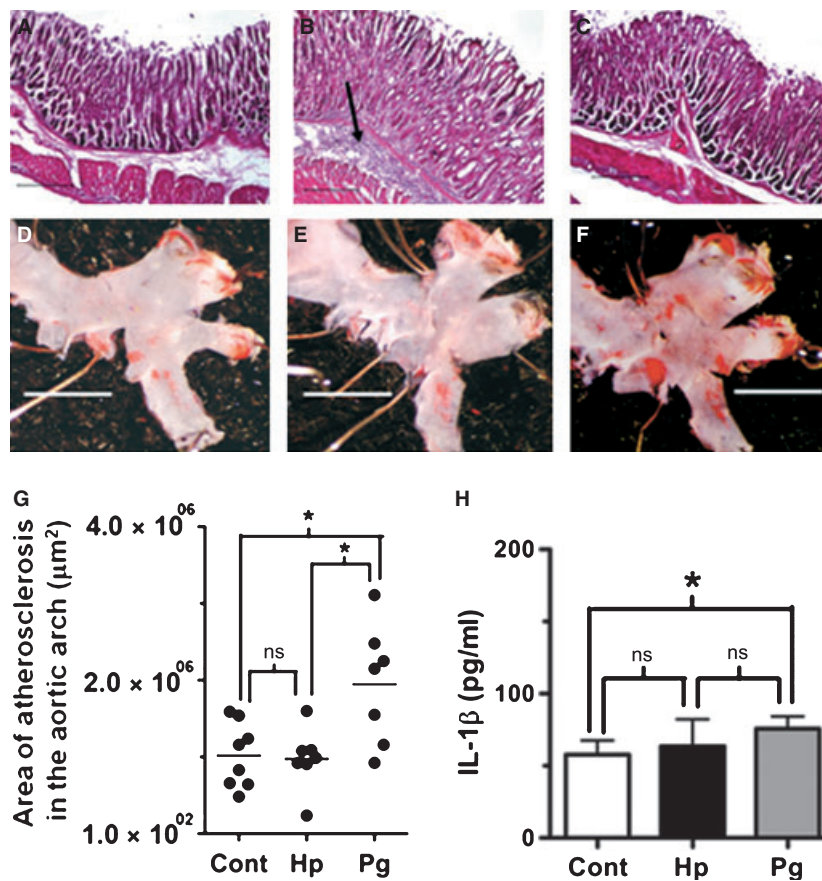


Figure 1 Chronic infection is established in apolipoprotein A-deficient ($\text{ApoE}^{-/-}$) mice challenged with *Porphyromonas gingivalis* and *Helicobacter pylori*, yet only *P. gingivalis*-infected mice present with acceleration of atherosclerosis. Stomach tissue from representative uninfected (A), *H. pylori*-infected (B), and *P. gingivalis*-infected (C) mice reveal that *H. pylori*-infected mice ($n = 8/\text{group}$) present with submucosal cellular infiltrate (arrow); representative micrographs of Sudan IV staining for lipids (red) on the intimal surface of the aortic arch region from uninfected (D), *H. pylori*-infected (E), and *P. gingivalis*-infected (F) mice show increased staining only in *P. gingivalis*-challenged mice, which was confirmed by morphometric analysis (G). Horizontal line denotes the group median and black circle represents an individual mouse. Serum levels of the proinflammatory cytokine interleukin-1 β (IL-1 β) as measured by enzyme-linked immunosorbent assay (H); * $P < 0.05$ by analysis of variance with Dunn's multiple comparisons; ns = not significantly different; scale bars in A–C = 25 μm , and D–F = 1 mm.

host cells and use both TLR2 and TLR4 for host cell signaling. However, the innate immune signaling pathways used by *P. gingivalis* are both host cell type and bacterial ligand specific. *P. gingivalis* lipopolysaccharide (LPS) has been demonstrated to activate host cells through both TLR2 and TLR4 and this may be dependent on the expression of various forms of *P. gingivalis* lipid A (Darveau *et al.*, 2004). The major and minor fimbriae proteins of *P. gingivalis* induce the expression of proinflammatory cytokines and use both TLR2 and TLR4 for this response (Hajishengallis *et al.*, 2004, 2006; Gibson *et al.*, 2008). The major fimbriae use TLR1 or TLR6 for cooperative TLR2-dependent activation of transfected cell lines. We have demonstrated that fimbriae bind to

TLR2 and signal through TLR2. Although fimbriae did not bind to TLR4 they were capable of signaling through TLR4 in the presence of MD2 (Davey *et al.*, 2008).

Cell-specific innate immune signaling is the result of multiple factors including the pattern recognition receptors expressed by these cells, the location of these receptors, the adaptors expressed by these cells, whether or not they are phagocytic or non-phagocytic cells, and how they process microbes or their antigens (Harari *et al.*, 2006). There also is evidence that tissue specificity can influence host cell signaling to microbial stimulation. We postulate that bacterial infection mediates inflammatory responses that involve specific innate immune pathways in

defined host cells and that this can impact inflammatory outcomes in chronic inflammation. In addition to promoting inflammatory responses to *P. gingivalis*, recent studies point to cell-specific innate immune pathways, which function to control persistence of the organism. We predict that activation of signaling cascades leading to inflammatory cytokine expression will proceed independently of pathways leading to bacterial uptake and the generation of bactericidal molecules. A number of investigators have examined possible immune evasion strategies of *P. gingivalis*, which could potentially contribute to bacterial persistence and chronic inflammation. These are discussed below in the context of the specific cell type in which this has been examined.

***P. GINGIVALIS* PERSISTENCE AND INFLAMMATION IN DENDRITIC CELLS**

Long-lived inflammatory cells such as dendritic cells, strategically poised along portals of entry, sample the local microenvironment and interact initially with *P. gingivalis* in the oral mucosa. Dendritic cells have a critical role in determining the nature of the immune reactions and in fine-tuning the balance between tolerance and the induction of inflammation. A number of studies have established that *P. gingivalis* can survive and replicate in dendritic cells. *P. gingivalis* expressing the major fimbriae are more efficient than fimbriae-deficient *P. gingivalis* in entering dendritic cells and in inducing a T helper type 1 inflammatory response (Jotwani & Cutler, 2004). *P. gingivalis* binds to dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) via the minor

fimbriae protein, is internalized and routed in large numbers to intracellular vesicles of dendritic cells (Zeituni *et al.*, 2009). These investigators also established that major fimbriae are immunostimulatory and the minor fimbriae are immunosuppressive and they proposed that the ability of *P. gingivalis* to uncouple dendritic cell maturation from the cytokine response could contribute to bacterial persistence. In addition, it is intriguing to postulate that *P. gingivalis* is trafficked to the atherosclerotic lesion via these immune cells including monocytes/macrophages or dendritic cells and that this may contribute to bacterial persistence *in vivo* (Wang *et al.*, 2007; Hajishengallis *et al.*, 2008b).

***P. GINGIVALIS*-INDUCED PLATELET ACTIVATION**

Platelet aggregation is important in the formation of atherosclerotic plaque (Ross, 1993). The expression of functional TLRs in platelets provides a possible mechanism by which platelets interact with bacteria and further highlights the importance of platelets in the immune and inflammatory responses. We recently demonstrated that in mice *in vivo* challenge with live *P. gingivalis* induced a platelet-dependent proinflammatory response and this response was significantly reduced in TLR2-deficient mice (Blair *et al.*, 2009). A role for fimbriae in *P. gingivalis*-mediated platelet aggregation was also confirmed using human platelets. As shown in Fig. 2, enhanced platelet aggregation was observed when platelets were incubated with wild-type *P. gingivalis* (381), but not with a major fimbriae mutant (DPG3). We also observed that wild-type *P. gingivalis*, but not DPG3, induced

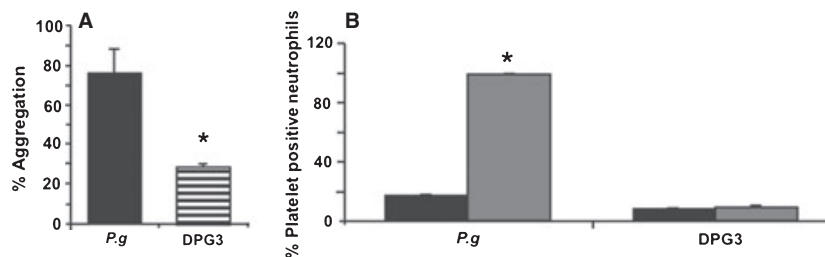


Figure 2 *Porphyromonas gingivalis* induces human platelet aggregation. (A) *P. gingivalis* 381 (*P.g*) or DPG3 (DPG3) at 10^8 colony-forming units (CFU) ml^{-1} , was added to a suspension of human platelets ($2 \times 10^8 \text{ ml}^{-1}$) and stirred continuously for 10 min in a PAP-4 aggregometer. The % aggregation relative to a collagen control is indicated. * $P < 0.001$ vs. collagen control. (B) *P. gingivalis* 381 (*P.g*) or DPG3 (DPG3) (10^8 CFU ml^{-1}) was added to suspensions of human whole blood (final platelet concentration, $2 \times 10^8 \text{ ml}^{-1}$) and stirred continuously in a whole blood aggregometer and formation of platelet–neutrophil aggregates was determined by flow cytometry. Black = no *P. gingivalis* addition; gray = + *P. gingivalis*. * $P < 0.001$ vs. unchallenged.

human platelet neutrophil aggregation *in vitro* (Fig. 2B). The ability of *P. gingivalis* to induce platelet aggregation via fimbriae-mediated stimulation may play an important role in *P. gingivalis*-mediated inflammatory responses leading to atherosclerosis. It has also been suggested that platelets may function to transport *P. gingivalis* to the atheroma, although bacterial persistence in platelets has not been examined in detail.

***P. GINGIVALIS* PERSISTENCE AND INFLAMMATION IN ENDOTHELIAL CELLS**

Local and distant chronic inflammatory lesions associated with *P. gingivalis* infection are initiated at the vascular endothelium (Moughal *et al.*, 1992). *P. gingivalis* invades endothelial cells, where it survives, replicates, and induces a proinflammatory response within these cells (Gibson *et al.*, 2006, 2008). In primary human coronary artery endothelial cells (HCAEC) of the coronary artery *P. gingivalis* can be found within autophagosomes and may use components of the autophagocytic pathway as a means to survive within these cells (Progulske-Fox *et al.*, 1999). *P. gingivalis* also appears to regulate host genes differently during invasion of human aortic endothelial cells (HAEC) (Chou *et al.*, 2005). Invasive *P. gingivalis* has also been reported to increase the adhesion of mononuclear leukocytes to HAEC (Roth *et al.*, 2007). We demonstrated in HAEC that *P. gingivalis* induces an IL-1 β response that is dependent on *P. gingivalis* invasion and precedes the monocyte chemoattractant protein-1 (MCP-1) and IL-8 response (Takahashi *et al.*, 2006). Stimulation of HAEC with purified *P. gingivalis* fimbriae did not stimulate IL-1 β but stimulated IL-8 and MCP-1. In contrast to TNF- α and IL-6, activation and release of IL-1 β requires two distinct signals (Mariathasan & Monack, 2007). The first signal can be triggered by TLR activation via nuclear factor- κ B activation, which leads to the synthesis of pro-IL-1 β . The second signal involves caspase-1 activation, which is controlled via the inflammasome, a cytosolic signaling complex. Activation and release of IL-1 β are both pathogen and host cell type-specific. Although *P. gingivalis* induces a potent IL-1 β response in endothelial cells and macrophages *in vitro*, how IL-1 β is induced in these cells, and how this modulates immune cell functions are not known.

We also demonstrated that *P. gingivalis* wild-type bacteria, but not the *fimA* mutant, stimulate TLR expression on the surface of the endothelium and that these primed cells respond to defined TLR-specific ligands (Yumoto *et al.*, 2005). Increased cell surface expression of TLRs on HAEC was not observed after treatment with purified fimbriae. Our results suggest that *P. gingivalis* modifies the levels of TLRs expressed on the surface of HAEC and that *P. gingivalis* infection can convert HAEC from TLR ligand hyporesponsive cells to TLR ligand hyper-responsive cells. Chronic and episodic stimulation of the endothelium with *P. gingivalis* may therefore sensitize the endothelium to microbial ligands or other endogenous TLR ligands and in this way contribute to the progression of atherosclerosis (Fig. 3).

***P. GINGIVALIS* PERSISTENCE AND INFLAMMATION IN MONOCYTES/MACROPHAGES**

Monocytes represent one of the primary cell populations present in chronic periodontal lesions and also play a role in foam cell formation in atherosclerosis. Gene expression profiling studies have demonstrated that macrophages respond differently to live *P. gingivalis* when compared with LPS or the FimA protein in the context of cytokine secretion and induction of intracellular molecules (Zhou *et al.*, 2005). *P. gingivalis* stimulation of human or mouse monocytes leads to the expression of TNF- α , IL-1 β , MCP-1, IL-6, IL-8, nitric oxide, and prostaglandin E₂ and this is dependent on expression of fimbriae (Gibson *et al.*, 2006). Expression of TNF- α and IL-6 is dependent on both TLR2 and TLR4. *P. gingivalis* fimbriae and LPS require lipid rafts for signaling and use similar yet distinct receptor complexes for interaction with host cells (Hajishengallis *et al.*, 2006). Intracellular survival of *P. gingivalis* is diminished in TLR2-deficient or CD11b-deficient macrophages, indicating that this pathogen manipulates complement and TLRs for persistence in macrophages (Wang *et al.*, 2007).

P. gingivalis and fimbriae or LPS have also been demonstrated to stimulate TLR2 surface expression on macrophages (Ukai *et al.*, 2008). Neither *P. gingivalis* fimbriae nor LPS was observed to upregulate TLR4 on mouse macrophages. Increased cell surface expression of TLR2 on mouse macrophages following stimulation with *P. gingivalis* fimbriae protein was associated with an increase in TNF- α following sub-

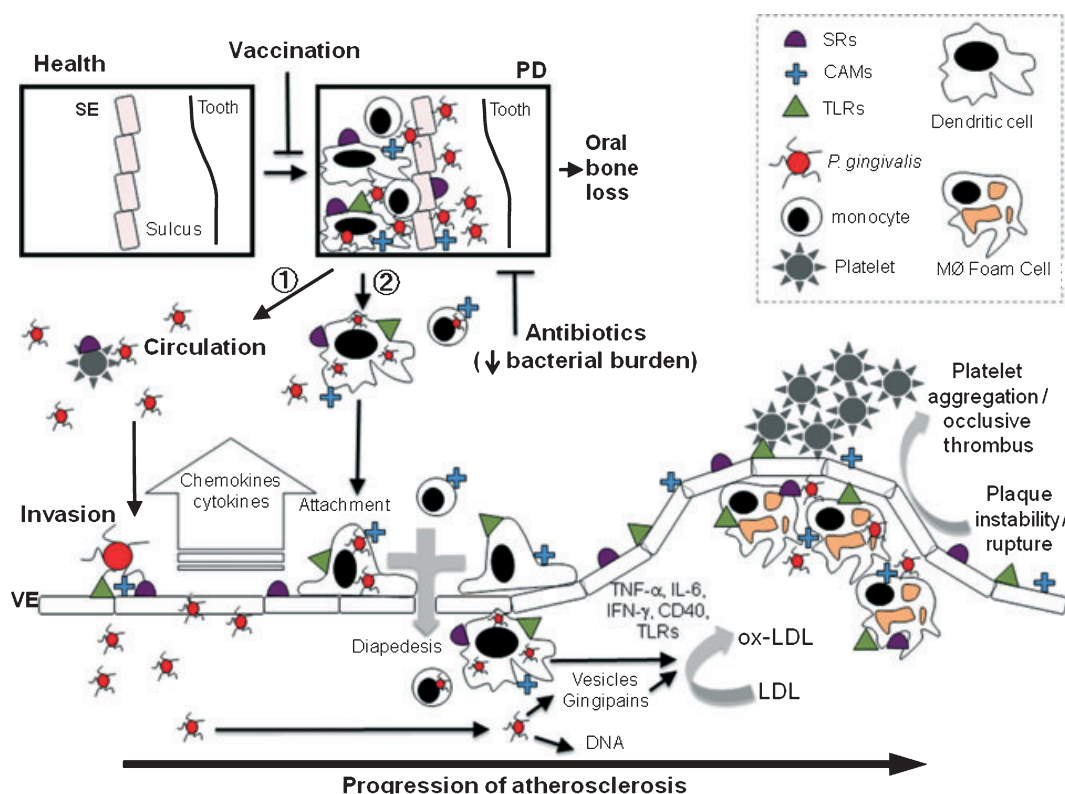


Figure 3 Model for *Porphyromonas gingivalis* systemic infection and acceleration of atherosclerosis. (i) Platelets, monocytes, and dendritic cells are depicted in circulation. (ii) Monocytes and dendritic cells are depicted in the oral mucosal lesion. Vaccination prevents oral colonization whereas antibiotics decrease the bacterial burden. Platelets, monocytes, dendritic cells, and foam cells are depicted at the atheroma. CAMs, cell adhesion molecules; IFN, interferon; IL, interleukin; M ϕ , macrophages; LDL, low-density lipoprotein; PD, periodontal diseases; SE, sulcular epithelium; SRs, scavenger receptors; TNF, tumor necrosis factor; VE, vascular endothelium.

sequent challenge with live *P. gingivalis*. Hence, similar to what was observed in endothelial cells, *P. gingivalis*-mediated stimulation of TLR expression on monocytes may sensitize these host cells to microbial ligands, or other endogenous TLR ligands, and in this way contribute to atherosclerosis progression. There is also evidence of cross-talk between endothelial cells and monocytes/macrophages resulting in enhanced inflammatory responses via the secretion of soluble mediators that have pleiotropic functions in inflammation.

Inflammation in macrophages that is induced by *P. gingivalis* also contributes to the formation of foam cells (Giacona *et al.*, 2004), one of the primary cells that make up the atherosclerotic lesion (Stary *et al.*, 1994; Libby, 2000). The accumulation of macrophages within the arterial wall sets the stage for progression of atheroma to more complicated plaques such as vulnerable plaques, i.e. those that are prone to rupture, that are thought to be responsible for occlu-

sive cardiovascular disease (Libby, 2000). Accumulation of lipids inside cells such as macrophages occurs by scavenger receptor (SR)-mediated uptake of modified LDL species (Moore & Freeman, 2006). The SRs are a broad group of surface expressed trans-membrane, multi-domain proteins that function as pattern recognition receptors. They are regulated on the surface of macrophages in response to stimulation, and demonstrate broad ligand specificity for polyanionic molecules. SRs also bind gram-negative and gram-positive bacteria, bacterial structures such as LPS and lipoteichoic acid, as well as advanced glycation end products.

CELL SPECIFICITY OF *P. GINGIVALIS*-INDUCED INFLAMMATORY RESPONSES EXAMINED *IN VIVO*

Although the focus of this review is on *P. gingivalis*-mediated chronic inflammation and atherosclerosis, it

is worth briefly discussing the cell specificity of *P. gingivalis*-induced inflammation in the context of other inflammatory outcomes of infection. A recent study examined the role of TLR2 in the control of *P. gingivalis* acute pulmonary infection (Hajishengallis *et al.*, 2008a). These investigators observed that TLR2-deficient mice exhibited reduced proinflammatory responses in the lung and had impaired clearance of *P. gingivalis* compared with wild-type mice, but that the levels of macrophages and polymorphonuclear cells in the lungs were similar in both strains of mice. These authors concluded that TLR2 plays an important role in clearance of *P. gingivalis* in acute lung infection. However, we and others have demonstrated that TLR2 mediates destructive effects in oral infection and oral bone loss (Gibson *et al.*, 2008) and in atherosclerosis (Madan & Amar, 2008; Hayashi *et al.*, 2010). Hence, TLR2 may play diverse and contextual roles in *P. gingivalis* infections exacerbating certain chronic inflammatory conditions (oral bone loss and atherosclerosis) while providing protection against acute conditions (pulmonary infection). This may also explain recent data in a mouse subcutaneous infection model in which mice deficient in the TLR adaptor proteins MyD88 and Toll-IL-1 receptor domain-containing adaptor-inducing interferon- β were shown to exhibit similar proinflammatory cytokine responses following challenge with *P. gingivalis* (Burns *et al.*, 2010). However, MyD88-deficient mice exhibited a deficiency in the ability to clear *P. gingivalis* systemic infection in this model in which the majority of cells encountering *P. gingivalis* following subcutaneous challenge are neutrophils. The role of TLR2 and MyD88 in promoting or clearing infection may therefore depend on the particular cell types present at the site of infection. These studies further highlight that activation of signaling cascades leading to inflammatory cytokine expression may proceed independently of pathways leading to bacterial uptake and the generation of bactericidal molecules, and may be related to the specificity of sites of bacterial colonization and inflammatory responses.

P. GINGIVALIS DISSEMINATION, PERSISTENCE AND INFLAMMATION LEADING TO ATHEROSCLEROSIS

The shift from periodontal health to periodontal disease involves a complex series of events that stem

from colonization with *P. gingivalis* in the subgingival plaque and development of an inflammatory nidus that leads to periodontal disease. We propose two non-exclusive mechanisms by which *P. gingivalis* oral infection can induce and maintain inflammation at sites distant from oral infection (Fig. 3).

1 *P. gingivalis* disseminates in circulation (blood or lymphatics) where it interacts with platelets and vascular endothelial cells. *P. gingivalis* binds to the vascular endothelium via major and minor fimbriae using a lipid raft-based cell receptor that is comprised of CAM/TLR/CD36. The infected vascular endothelium responds by the production of cytokines, chemokines, and surface molecules. *P. gingivalis* and antigens in the vascular intima serve as part of the stimulus that drives naive immune cell localization/activation. In support of this pathway we previously reported that *P. gingivalis* could be detected by 16S polymerase chain reaction in both blood and aortic tissue during the oral infection period, but not at later time points after bacterial challenge (Gibson *et al.*, 2004). Polymerase chain reaction analysis also revealed detection of the *P. gingivalis* *fimA* mutant in both blood and aortic tissue, although the *fimA* mutant did not induce atherosclerosis. Although we have not detected *P. gingivalis* by culture techniques in aortic tissue, this does not exclude the possibility that viable organisms are present. Based on our earlier studies in other mouse model systems we propose that *P. gingivalis* can localize to atherosclerotic lesions. Using a subcutaneous chamber model we have previously determined that *P. gingivalis* can be cultured from chamber exudates, heart, liver, kidney, and spleen 3 days after infection (Genco *et al.*, 1991, 2002; Mydel *et al.*, 2006). Using the chamber model, we established that *P. gingivalis* infection results in significant bacteremia with detection of 2×10^3 – 3×10^3 colony-forming units of *P. gingivalis* in blood samples reported, as well as high levels of LPS (Mydel *et al.*, 2006). Likewise, other investigators have detected viable *P. gingivalis* in the lung following intratracheal inoculation (Hajishengallis *et al.*, 2008a). It therefore appears that *in vivo* *P. gingivalis* can survive in blood and host tissues. What remains to be determined is if *P. gingivalis* colonizes aortic tissue for extended periods of time leading to inflammatory cascades or if *P. gingiva-*

lis transiently colonizes and sensitizes the endothelium via stimulation of innate immune receptors, which then respond to other endogenous ligands.

- 2 *P. gingivalis* enters immune cells such as monocytes/macrophages or dendritic cells in the diseased/inflamed oral mucosal lesion, these cells then leave the inflamed tissues of the oral cavity, enter the circulation, localize, and diapedese into the vascular intima at sites of activated vascular endothelium (pathogen-activated or activated by inflammation associated with atherosclerosis). Rupture-prone unstable plaques in particular, are infiltrated with high numbers of activated SIGN⁺ myeloid dendritic cells (Soilleux *et al.*, 2002). Although the sources of these dendritic cells in atherosclerosis are not clear, lesions obtained from patients with chronic periodontitis contain an intense infiltrate of SIGN⁺ dendritic cells (Jotwani *et al.*, 2003).

Immune cells activated by *P. gingivalis* drive modification of low-density lipoproteins to forms such as ox-LDL that are taken up by macrophages, leading to foam cell formation. These atherosclerotic lesions progress, develop a necrotic core, and when ruptured form an occlusive thrombus leading to a downstream ischemic event. It has been shown experimentally that vaccination to prevent oral bone loss also mitigates *P. gingivalis* acceleration of atherosclerosis. Use of antibiotics as part of aggressive periodontal clinical treatment effectively reduces the overall pathogen burden at the site of treatment and would result in lowering the risk for development of atherosclerosis.

FUTURE DIRECTIONS

Although epidemiological and experimental studies link infectious agents with the development of inflammatory atherosclerosis, the precise mechanisms contributing to this link have remained elusive. Recent seminal studies with the oral pathogen *P. gingivalis* have defined pathogen-specific persistence strategies and the modulation of cell-specific innate immune inflammatory pathways required for initiation and maintenance of *P. gingivalis* induced chronic inflammation at sites distant from oral infection. Despite these significant studies a number of questions remain (Table 1). We encourage future studies to examine both *P. gingivalis* and host-cell-specific path-

Table 1 Questions that remain and areas that should be pursued in future studies

Pathogen
Does <i>P. gingivalis</i> disseminate to the atherosclerotic lesion?
Does <i>P. gingivalis</i> colonize within the atherosclerotic lesion?
What immune evasion strategies allow <i>P. gingivalis</i> to survive?
Host
What host cells are used for innate immune signaling?
What proinflammatory mediators are required?
Are there different pathways for bacterial clearance vs. inflammatory mediator production?
Overall
Are there differences in pathogen-mediated and host-mediated mechanisms required for oral colonization and inflammation vs. colonization and inflammation at sites distant from infection?

ways for induction of inflammatory cascades and bacterial persistence. These studies will advance our understanding of the roles of specific immune cells that participate in chronic inflammation and atherosclerosis in response to oral infection with *P. gingivalis*, and will provide a promising avenue for novel therapies for chronic inflammatory diseases induced by bacterial infection.

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