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Porphyromonas gingivalis–dendritic cell interactions: consequences for coronary artery disease

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An estimated 80 million US adults have one or more types of cardiovascular diseases. Atherosclerosis is the single most important contributor to cardiovascular diseases; however, only 50% of atherosclerosis patients have currently identified risk factors. Chronic periodontitis, a common inflammatory disease, is linked to an increased cardiovascular risk. Dendritic cells (DCs) are potent antigen presenting cells that infiltrate arterial walls and may destabilize atherosclerotic plaques in cardiovascular disease. While the source of these DCs in atherosclerotic plaques is presently unclear, we propose that dermal DCs from peripheral inflamed sites such as CP tissues are a potential source. This review will examine the role of the opportunistic oral pathogen *Porphyromonas gingivalis* in invading DCs and stimulating their mobilization and misdirection through the bloodstream. Based on our published observations, combined with some new data, as well as a focused review of the literature we will propose a model for how *P. gingivalis* may exploit DCs to gain access to systemic circulation and contribute to coronary artery disease. Our published evidence supports a significant role for *P. gingivalis* in subverting normal DC function, promoting a semimature, highly migratory, and immunosuppressive DC phenotype that contributes to the inflammatory development of atherosclerosis and, eventually, plaque rupture.

Keywords: *dendritic cells*; *periodontitis*; *atherosclerosis*; *Porphyromonas gingivalis*; *DC-SIGN*

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The pathological manifestations of chronic periodontitis (CP), namely, destruction of the soft and hard tissues that support the dentition culminating in tooth loss, have been previously described, as has the high prevalence of the CP in the general adult population (1, 2). Also well documented is the specific role of the anaerobic Gram-negative species, *Porphyromonas gingivalis*, in infection of the tissues around the dentition in CP and in initiation of CP (3). *P. gingivalis*, along with two other species, *Tannerella forsythia* and *Treponema denticola*, comprise the so-called red complex of pathogens (4) that function cooperatively within the subgingival plaque of CP (1, 2, 5–7). *P. gingivalis* has been associated with several important systemic diseases such as cardiovascular disease, rheumatoid arthritis, preterm birth weight, and diabetes mellitus (8, 9). Our group is particularly interested in the influx and efflux of various dendritic cells (DCs) in response to *P. gingivalis* and their role in local and systemic inflammatory processes. DCs are very active antigen-capture cells when immature. When DCs mature, they become potent antigen-presenting cells and are very efficient at stimulating T-naïve cells to differentiate into T-cell effectors [reviewed in Steinman (10), Cutler (11)]. DCs have been implicated in a number of allergic and inflammatory diseases in the periphery [reviewed in Velazquez and Teran (12)] including atherosclerosis (13, 14). Being central to the development of immunologic memory and tolerance (15), the normal immunologic function of DCs is to patrol the periphery, capture infecting microbes and then migrate out to the secondary lymphoid organs where they can initiate and regulate the adaptive immune response. DC functions in this regard are tightly regulated and depend on the activation signals that DCs receive in the periphery [reviewed in Cutler and Jotwani (16)]. These signals include inflammatory

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cytokines, chemokines, as well as pathogen-associated molecular patterns (PAMPS) of bacteria. The development of gingivitis and periodontitis involves the influx and efflux of different DC subsets at distinct stages of disease (Fig. 1). Dendritic Langerhans cells (LC) infiltrate the gingival epithelium in gingivitis and then efflux into the lamina propria in CP, where they begin to undergo maturation (17). Dermal dendritic cells (DC-SIGN+) increase in the lamina propria in CP and become localized toward the lymphatics and vasculature (18–20). LCs have been implicated in both the initiation and regulation of contact-hypersensitivity responses in mice (21) [reviewed in Igyarto and Kaplan (22)], while dermal DCs have been implicated in many other inflammatory diseases including rheumatoid arthritis and inflammatory bowel disease (23). This review will focus

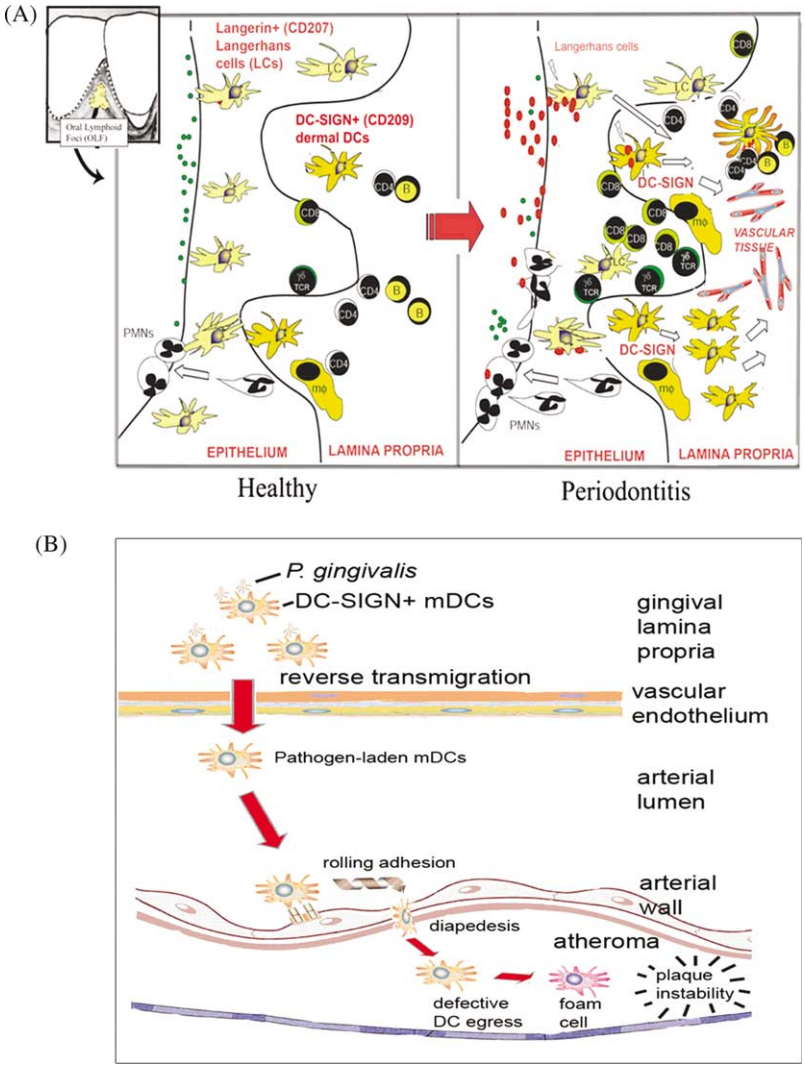

Fig. 1. Schematic representation of how *P. gingivalis*-laden dendritic cells may promote atheroma formation and maturation. (A) Representation of the oral lymphoid foci, its organized inflammatory infiltrate. The left panel depicts the healthy oral biofilm in the gingival crevice, comprised predominantly of Gram-positive bacteria (green dots). Healthy gingival tissue is infiltrated with numerous Langerhans cells in the epithelium, with sparse dermal dendritic cells in the lamina propria. As disease progresses, the oral biofilm changes to a predominantly Gram-negative subgingival flora (red dots). In response, a dramatic loss (efflux) of Langerhans cells occurs from the epithelium toward the lamina propria. Also observed is an influx of myeloid-derived DC-SIGN+ dermal dendritic cells (DCs) into the lamina propria. Present are neutrophils, macrophages (mϕ), B-cells, and CD4+ and CD8+ T-cells. The DCs form immune conjugates with CD4+ T-cells and also mobilize toward the vasculature. (B) Hypothetical model in periodontitis, showing mobilization of *P. gingivalis*-laden DC-SIGN+ myeloid DCs (mDCs) in the gingival lamina propria. These mDCs undergo reverse transmigration through the vascular endothelium. Once in the circulation, mDCs carrying *P. gingivalis* attach to endothelial integrins via DC-SIGN and, after rolling adhesion, undergo diapedesis between endothelial cells. As the atheroma continues to mature and DCs contribute to the foam cells and release MMP-9, the atheroma becomes highly unstable and, eventually, thrombus formation occurs.

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on *P. gingivalis* and DCs and their respective contributions to the development (and instability) of atherosclerotic plaques.

Atherosclerosis and microbes

Atherosclerosis (ATH) is a progressive disease characterized by the accumulation of lipids, fibrous elements, and inflammatory cells in the large arteries. ATH constitutes the single most important contributor to the growing worldwide burden of cardiovascular disease. Only about 50% of patients with ATH have currently identified risk factors (24). This suggests how little we know about ATH risk. Inflammation in the arterial vessel wall is particularly important in the development of ATH. Four mechanisms have been proposed for how bacterial pathogens may induce or accelerate ATH [reviewed in Gibson et al. (25)]. These include: (i) direct invasion of the vascular endothelium by pathogens in the blood, (ii) immunological sounding, (iii) molecular mimicry, and (iv) pathogen trafficking of microbes within leukocytes in peripheral blood.

Of particular relevance to atherogenesis are bacterial species that infect and survive within endothelial cells and within migrating leukocytes [reviewed in Rackley (26)]. A prevailing hypothesis is that, regardless of their viability status, bacteria release PAMPs that serve as agonists for TLRs, thus activating inflammatory leukocytes and endothelial cells, and can contribute to the development of ATH (27). Large population studies support the role of bacterial species in ATH (28, 29). However, the results of clinical trials using antibiotics to treat cardiovascular disease have been disappointing (30–33). Many atherogenic bacteria, including *P. gingivalis*, are intracellular pathogens (34, 35). An apparent consequence of this is that these pathogens are less susceptible to antibiotics when sequestered inside host cells. *P. gingivalis* is 100-fold more resistant to moxofloxocin, 10-fold more resistant to clindamycin and metronidazole when inside host epithelial cells (36). Clindamycin- and azithromycin-resistant *P. gingivalis* isolates have been identified in human subjects with CP (37).

P. gingivalis has also been identified in human ATH plaques (38), as have other atherogenic bacteria such as *Chlamydophila pneumoniae* and *Helicobacter pylori* (27, 39, 40). Experimental infection with *P. gingivalis* accelerates ATH in animal models (35, 41). The FINRISK 1992 cohort study of 6,051 individuals implicates exposure to *P. gingivalis* or endotoxin in increased risk for cardiovascular diseases (42). A recent meta-analysis indicated that the level of systemic bacterial exposure in CP mediates ATH risk (43, 44). Another meta-analysis studied human cohort studies, case-control studies, and cross-sectional studies and concluded that CP is a significant risk factor for developing coronary artery disease (CAD) (44). The degree of increased risk of CAD conferred by CP appears comparable to smoking (45) and elevated serum triglycerides (46). Viable and invasive *P. gingivalis*, though in a dormant state, have been cultured from human ATH plaques (38). This has not been shown with other atherogenic bacteria such as *C. pneumoniae* and *H. pylori*, which have only been identified by DNA-based methods (27, 39, 40). Overall, several infectious agents have been shown to be disseminated by pathogen trafficking leukocytes, they include *Streptococcus pyogenes* (47), *C. pneumoniae* (48), and *Listeria monocytogenes* (49).

P. gingivalis virulence and targeting of dendritic cells (DCs)

P. gingivalis is a an amino acid fermentor with an absolute requirement for hemin (2). The bacteria utilizes its many virulence factors to fulfill its complex nutritional requirements, while still enabling it to evade and even modulate the host immune system (1, 2, 5). Several virulence factors including the polysaccharide capsule, fimbriae, proteases for opsonins C3, proteases for IgG, gingipains, bacterial lipopolysaccharides (LPS), toxins, and hemagglutinins enable *P. gingivalis* to persist in the oral mucosa and help facilitate some of the physiopathology of CP (19, 50–56).

There are two fimbriae that are essential adhesins for the invasion and colonization of the oral mucosa by *P. gingivalis*. These adhesins, termed the major and minor fimbriae, are distinct antigenically by amino acid composition and by size (57, 58). The major fimbriae form long projections from the bacteria and have been shown in most reports to facilitate their adhesion to and invasion of the host cells. The major fimbriae is a 41 kDa protein, encoded by the *fimA* gene (59), and many of its cellular receptors have been identified as either the β-1 integrins (CD29) (60, 61) or the β-2 integrins (CD18) (62–64). The minor fimbriae (though much shorter) are comprised of a 67 kDa protein encoded by the *mfa1* gene (57). We have recently shown that the minor fimbriae targets DC specific ICAM-3 grabbing non-integrin (DC-SIGN or CD209) on monocyte-derived DCs for entry (5). DC-SIGN is a type II membrane protein in which the extracellular domain consists of a stalk that promotes tetramerization (65). It contains a C-terminal carbohydrate recognizing domain (CRD) that belongs to the C-type lectin superfamily (65). Early studies by Feinberg et al. showed that the DC-SIGN CRD preferentially binds to the high-mannose N-linked oligosaccharides GlcNAc (N-acetylglucosamine) and Manα1-3[Manα1-6] Man (mannose) (65). Furthermore, Appelmelk et al. showed that DC-SIGN also binds to fucose-containing Lewis blood antigens (66). Guo et al. utilized an extensive glycan array and showed that DC-SIGN will bind high mannose-containing glycans or glycans that contain terminal fucose residues (67). Previous studies showed that DC-SIGN is used by

microorganisms such as *Neisseria gonorrhoeae* (68), *Mycobacterium tuberculosis* (66, 69, 70), *Mycobacterium leprae*, HIV (71), *H. pylori* (66), and *P. gingivalis* (5) to target DCs for entry and immune suppression.

Pathological consequences of DC-SIGN targeting

The ability of minor fimbriae to specifically target DC-SIGN on DCs has significant pathological and immunological repercussions. Periodontitis lesions contain an intense infiltrate of DC-SIGN+ DCs (17, 72). DCs in the lesions become activated and appear to mobilize toward the capillary-rich lamina propria (17). We propose that infection of DCs by *P. gingivalis* triggers reverse transmigration of infected gingival DCs into circulation and these may contribute to the pathogenesis of ATH (Fig. 1). Evidence exists for the presence of activated DC-SIGN+ myeloid DCs in rupture prone unstable plaques (13, 73). The sources of these DC-SIGN+ plaque DCs are not clear, but presumably may include DCs from inflamed peripheral tissues. Other sources of DCs may include CD14+ CD16− monocytes, CD14low CD16+ monocytes (74) that differentiate into DCs *in situ* (5). DCs that infiltrate rupture-prone atherosclerotic plaques express atherogenic markers including C1q (a classical complement pathway component involved in apoptotic cell clearance); HSP60 and HSP70 (chaperone proteins involved in autoimmune responses); chemokine receptors CCR2, CCR5, CX3CR1; and chemokines CXCL16, CCL19, and CCL21 (involved in DC transmigration and leukocyte homing) (74–76). Also expressed are DC maturation markers CD40, CD80, and CD86 (73, 77). Matrix-metalloproteinase-9 (MMP-9), which are highly expressed in vulnerable regions of the atherosclerotic plaques and suggested to be causally involved in plaque rupture (78), is produced by leukocytes including DCs. The *P. gingivalis* LPS is a particularly potent inducer of MMP-9 but not TIMP-1 by DCs, suggesting that *P. gingivalis* induces an MMP-9/TIMP-1 imbalance in DCs (79). Our published data indicated that fimbriated strains of *P. gingivalis* infect DCs and induce atherogenic biomarkers *in vitro*, but the mechanisms are not presently clear (5).

Immunological consequence of targeting DC-SIGN on dendritic cells (DCs)

DC-SIGN targeting by minor fimbriae results in a dampening of the maturation status and the inflammatory cytokine profile of DCs. Conversely, removal of the minor fimbriae results in robust DC maturation coupled with a strong proinflammatory cytokine response (5). This regulation of DC immunogenic functions based on minor fimbriae expression extends to the T-cell effector response elicited by DCs. In DC-CD4+ T-cell coculture experiments, *P. gingivalis* strains expressing solely the minor fimbriae induced DCs to prime T-cells into a Th_2 effector phenotype, whereas, strains expressing solely the major fimbriae induced DCs to prime T-cells into a Th_1 effector phenotype (5). Intriguingly, the wild-type strain was able to stimulate a mixed or anergic T-cell effector phenotype (5). Furthermore, *in vitro* studies in the presence of DC-SIGN targeting agonists (e.g. mannan from *S. cerevisiae* or glycosylated HIV gp120) resulted in a diminished association of minor fimbriated strains with cells (5). When the wild-type strain of *P. gingivalis* was cocultured with glycosylated HIV gp120, we observed a dramatic uncoupling of DC maturation from DC inflammatory cytokine secretion (5). The presence of HIV or even *Candida albicans* (etiological agent of oral thrush) might act synergistically with *P. gingivalis* to further exasperate this uncoupling and act to greatly diminish DC maturation.

Mechanisms of DC mobilization, access to peripheral blood

Experimental studies demonstrate the important role that chemokines and chemokine receptors play in trafficking of leukocytes to and invasion of the arterial wall in ATH (80–84). Immature DCs express inflammatory chemokine receptors (Table 1) that direct their migration into infected tissues. In response to capture of antigens or to TLR-mediated recognition of microbes, DCs undergo a process called functional maturation in which they downregulate inflammatory chemokine receptors and upregulate homeostatic chemokine receptors (Table 1). This directs DC migration out of the tissues toward lymph nodes. When the DC maturation process is disrupted, as occurs in DC-SIGN ligation (5), activated DCs ostensibly undergo reverse transmigration into the blood (Fig. 1), which can lead to systemic inflammation. This process contributes to the initiation, progression, and instability of arterial plaque in patients with CAD. Recent studies have investigated the presence of blood DCs in patients with CAD, but the results are controversial (85, 86). Shi et al. found that the total peripheral blood CD11c+DCs were significantly higher in patients with CAD compared to healthy controls (85). Conversely, Yilmaz et al. found a decrease in circulating DCs in patients with CAD (86). However, the patient population and markers used for DC isolation were different. None of the studies reported the presence of CP on these patients, which might have affected these conflicting findings. An attempt has been made to correlate the presence of blood DCs as a risk factor for CAD (87). Further, much speculation has been made about the source of these DCs that infiltrate the atheromas.

Fully matured DCs lose their ability to uptake and process antigens, stop migrating, and express CD83 and other molecules involved in antigen presentation to T-cells (88). Full maturation of DCs also results in loss

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Table 1. Summary of results of immunohistochemistry (IHC), cDNA microarray, and qRT-PCR analyses of MoDCs and gingival tissues

Chemokine receptors (their ligands)	Class	*P. gingivalis* pulsed MoDC (fold change)	Chronic periodontitis gingival tissue (fold change)
CCR2 (CCL2/MCP-1, CCL7/MCP3, CCL8/MCP2, CCL13/MCP4)	Inflammatory	5.7 × ↑[e] 10 × ↑[b]	2,000 × ↑[e]
CXCR6 (CXCL16 or SR-PSOX)	Inflammatory	2 × ↑[e]	300 × ↑[e]
CCR5 (CCL3 or MIP-1α, CCL4 or MIP-1β, CCL5 or RANTES, CCL11, CXCL8 or IL-8)	Inflammatory	12 × ↑[b]	20 × ↑[c]
CXCR3 (CXCL10)	Inflammatory	NT[a]	2.2 × ↑[d]
CX3CR1 (CX3CL1)	Inflammatory	NT[a]	NT[a]
CCR4 (CCL22)	Homeostatic	NT[a]	2.3 × ↑[d]
CCR6 (MIP-3α)	Homeostatic/ Inflammatory	NT[a]	10 × ↑[e]
CCR7 (CCL19 or MIP-3β, CCL20 or LARC)	Homeostatic	3 × ↑[b]	25 × ↑[e]

[a]NT, not tested.
[b]cDNA microarray (GEArray, Superarray) of *P. gingivalis* pulsed MoDC (3 h) or gingival tissues from diseased vs. control patients, normalized vs. β Actin.
[c]# cells/per field (IHC), 11 healthy vs. 11 control.
[d]Affymetrix chip results, normalized against internal standard.
[e]qRT-PCR results normalized against GAPDH.

of expression of many endocytic receptors, including DC-SIGN; thus, expression of DC-SIGN is one indicator that DC are not fully mature (89). There is evidence that a particular subset of blood DCs expressed DC-SIGN and that this receptor may be involved in the uptake and dissemination of HIV (90). Engering et al. showed that DC-SIGN+ blood DCs are able to stimulate proliferation of allogeneic T-cells, as well as infect these T-cells in trans (90). Potentially, DC-SIGN+ blood DCs can disseminate pathogens, increase systemic inflammation, and contribute to plaque instability.

Environmental regulation of fimbriae: possible role in systemic immunosuppression, dissemination of *P. gingivalis*

Wu et al. discovered that the major and minor fimbriae are regulated by a two component regulatory system termed FimS/FimR (91). It was also determined that while FimR binds directly to *mfa1*, it will only bind to the first gene of the *fimA* gene cluster, *pg2130* (91, 92). Moreover, this two component regulatory system responds to environmental cues like heme and temperature (91). However, it is still not clear how these adhesive fimbriae are regulated *in vivo*. The possibility of major and minor fimbriae being differentially regulated in response to different environmental cues or stimuli may allow this organism to modulate the immune system to expand its ecological niche. Moreover, our observations of intact bacteria inside DC-SIGN rich vesicles might explain the dissemination of this organism to atherosclerotic plaques (5). We propose here that *P. gingivalis* interacts with DC-SIGN on dermal DCs from the gingiva mucosa (unpublished observation). This interaction facilitates uptake of *P. gingivalis* and immunomodulation of normal DC functions. Directed migration is disrupted and DCs then migrate through the endothelium instead of the lymphatic system (Fig. 1). Once in the endothelium, the DC undergoes partial maturation resulting in adherence to the endothelium and recruitment of other leukocytes. Soilleux et al. previously described the presence of immature (lacking CD83 but expressing HLA-DR and LAMP) DC-SIGN$^+$ DCs on atherosclerotic plaques (73). Strikingly, this maturation profile is very similar to our results with minor fimbriated strains (Pg381 and DPG-3) and DCs (5). Thus, *P. gingivalis* infected DCs may exit into the bloodstream and migrate to the site of developing atheroma, where they adhere to and invade the arterial endothelium. Recent reports suggest that *P. gingivalis* is able to spread from infected epithelial, endothelial, and smooth muscle cells to new host cells where it multiplies (93). These mechanisms might explain how an oral opportunistic pathogen is able to disseminate throughout its host and potentially facilitate the formation of atherosclerotic plaques.

Conflict of interest and funding

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