A Review on the Porphyromonas gingivalis pathogens and the participatory function of caspase-1 in atherosclerosis process

Rami Al Batran[1], Fouad Al-Bayaty*[1]

1Center of Studies for Periodontology, Faculty of Dentistry, Universiti Teknologi MARA (UiTM), 40450 Shah Alam, Selangor Darul Ehsan, Malaysia.
drfouadhm@yahoo.com

ABSTRACT

Although an association between periodontitis and cardiovascular diseases has been suggested, the role of *Porphyromonas gingivalis* (*Pg*) in cardiovascular diseases is not clear. Studies in humans support a role for the oral pathogen *Pg* in the development of inflammatory atherosclerosis. *Pg* a key pathogen in periodontal disease promotes atherosclerosis in apolipoprotein E-deficient *(apoe⁻/⁻)* mice. Previous studies have provided indirect evidence that *Pg* is capable of activating the enzyme caspase-1 *in vitro,* while others studies carried out to confirm the role of caspase-1 in atherosclerosis in *apoe⁻/⁻* mice. Whitman lab study provides direct evidence that *Pg* is capable of activating caspase-1, and that *Pg* is capable of gaining access to and localizing at, sites of lesion development following an oral challenge. Oral infection with *Pg* exacerbated the development of atherosclerosis in the aortic root of male mice competent for caspase-1 as compared to those deficient in the enzyme.

Keywords: Porphyromonas gingivalis, periodontal disease, atherosclerosis, caspase-1

1. Introduction

Periodontitis is a pathological destructive inflammatory condition that affects the supporting structures of teeth, Connective tissue attachment, and alveolar bone (Breivik *et al*, 2000; Breivik *et al,* 2005; Al-Bayaty *et al*, 2011). Degradation of the periodontal tissues in periodontal disease results from inflammatory reactions primarily derived from interactions between the hosts immune system and subgingival bacterial colonies (Al-Bayaty *et al*, 2010). It is thought to be caused by the accumulation of bacteria on tooth surfaces in the periodontal pockets. Despite identification of over 500 different bacterial species in the oral cavity, only a relative few organisms are linked to periodontal disease; however, *Pg* is the most common organism linked to adult forms of periodontal disease. Over the past 10 years, mounting evidence has accumulated supporting a role for periodontal disease and infection, with *Pg* as a potential risk factor for several systemic diseases, including diabetes, preterm birth, heart disease, and atherosclerosis (Genco *et al*, 1996). *Pg*, a black-pigmented gram-negative anaerobic bacterium, is recognized as the most important causative organism in adult periodontitis (Lamont *et al*, 1998). *Pg* possesses a number of bioactive molecules on its cell surface that contribute to its pathogenesis, such as cytoplasmic membranes, peptidoglycans, outer membrane proteins,

lipopolysaccharides (LPS), capsules, caspases and fimbriae which can induce excessive cytokine production. *Pg* LPS is an important pathogenic component in the initiation and development of periodontal disease (Wang *et al,* 2002).

On other hand, Atherosclerosis is a chronic inflammatory disease characterized by sub-endothelial accumulation of inflammatory cells and lipids, which collectively contribute to occlusive disease or to less occlusive plaques at high risk for disruption. The activation of endothelial cells at atherosclerotic lesion-prone sites in the arterial tree results in the up-regulation of cell adhesion molecules and chemokines, which mediate the recruitment of circulating monocytes. Accumulation of monocytes, monocyte-derived phagocytes and T cells contribute to chronic inflammation and atherosclerosis. (Swirski *et al,* 2006) Epidemiological studies in humans and studies of atherosclerosis in mouse models support a role for infectious agents in inflammatory atherosclerotic plaque accumulation. Infectious agents can induce cellular and molecular changes characteristic of inflammatory processes observed in atherosclerosis. Several studies have implicated the induction of an inflammatory response by infectious agents as a possible mechanism linking infection to the acceleration of atherosclerosis (Haraszthy *et al,* 2000).

Evidence in humans suggesting that infection with *Pg* and periodontal disease predisposes to atherosclerosis is derived from studies demonstrating that periodontal disease pathogens reside in the walls of atherosclerotic vessels. Seroepidemiological studies demonstrating an association between the pathogen-specific antibodies and atherosclerosis (Libby *et al*, 2002). Recent studies reported that invasive bacteria are required for the acceleration of atherosclerosis, and that there is an innate immune response directed to invasive *Pg*, as demonstrated by the increased expression of innate immune receptors in the aorta of hyperlipidemic mice following oral challenge with *Pg* (Haraszthy *et al,* 2000). Complementary and alternative medicine (CAM), including herbal medicine, is popular in the general population worldwide (Al-Bayaty et al, 2012; Al-Bayaty et al, 2008). A number of herbs or plants with potent therapeutic components have been investigated against hyperlipidemia and anti oxidant such as andrographolide (Al Batran et al., 2013a; Al Batran et al., 2013b).

The goal of this review is to discuss specifically the association between periodontal disease and atherosclerosis, with particular emphasis on the putative mechanisms linking *Pg* participatory function of caspase-1 in atherosclerosis process.

2. P. gingivalis infection and atherosclerosis

Recent epidemiological data suggest that periodontal disease is an important risk factor for coronary heart disease, Proposed explanations include transient bacteremia with spread of infection from the oral cavity; endothelium injury by circulating oral microbial toxins; and systemic inflammation triggered by oral microorganisms. *Pg* infection causes local inflammation with gingival ulceration and vascular changes, which increase the incidence and severity of transient bacteremias with gingival trauma (Silver *et al,* 1977). Colonization of the subgingival region is due to the ability of the bacterium to adhere to any available substrate, such as extracellular matrix proteins, epithelial cells, and bacteria that have already established themselves as a biofilm on the tooth surface (Mysak *et al*,

2014). Secreted or cell-bound enzymes, toxins, and hemolysins play a significant role in the ability of the organism to spread throughout the host and in tissue destruction as it attacks host extracellular matrix proteins, cell adhesion molecules, and protein defenses secreted by the immune system, such as cytokines (Bodet *et al*, 2006).

Recent in vitro studies have demonstrated that *Pg* can adhere to and invade endothelial and coronary artery smooth muscle cells (Dorn *et al*, 1999). This suggests a highly efficient host endocytic uptake of these surface-adherent organisms and the ability of *Pg* to persist within endothelial cells and potentially to alter their integrity. It is conceivable that movement of this infection into the systemic circulation with frequent bacteremia could cause a chronic inflammatory insult to the vasculature and contribute to the initiation and progression of atherosclerosis lesions (Mattila *et al*, 1998; Offenbacher *et al*, 1999). It is possible that *Pg* is able to gain access to sites of atherosclerotic lesion development, potentially contributing to the progression of the disease.

Atherosclerotic events in humans are not usually detected until the advanced stages, long after they become clinically relevant (Glagov *et al*, 1987). Thus, studying human atherosclerotic events in their later stages does not provide insight into how atherosclerotic lesions develop and progress in their earliest stages. To this end, the mouse has become a powerful tool in investigating biology and molecular pathways involved in the formation atherosclerotic lesions at their earliest stages. Cholesterol is essential in all mammalian cells for proper cellular functions. It is either synthesized in the endoplasmic reticulum, or derived from the diet where it circulates through the body in the bloodstream (Tortora *et al*, 2003). Cholesterol is insoluble in blood and therefore has to be bound to water-soluble proteins, known as lipoproteins, to be transported in the circulatory system throughout the body (Vance *et al*, 2002). Lipoproteins rely on apolipoproteins (apos) associated with their surface to accurately target lipoproteins to sites of metabolism and removal (Vance *et al*, 2002). Apos function by mediating the interaction of lipoproteins with enzymes, transfer proteins and cell surface receptors (Tortora *et al*, 2003). Apolipoprotein E (apoE) associates with the surface of plasma lipoprotein particles and mediates the interaction with cell surface receptors. ApoE is the primary ligand for low density lipoprotein (LDL) receptor-mediated removal of lipoprotein remnants from the circulation (Plump *et al*, 1995). Therefor ApoE is essential for the removal of lipoproteins and their cargo from the circulation via the LDLr and the *apoe⁻/* mouse model have been invaluable in giving insight into many human disease processes, This model possesses several advantages, such as the ability to perform genetic manipulations using transgenic and gene-targeting technology, they are easy to breed, have a short generation time, and inbred strains are readily available. The ability of the *apoe⁻/* mouse model to clear plasma lipoproteins is impaired, causing the mice to become hyperlipidemic (Plump *et al*, 1995).

2.1 Systemic infection

Using an *in vivo* mouse model, Li *et al* (Li *et al*, 2002) tested the hypothesis that a long-term systemic circulatory challenge with *Pg* can promote and accelerate the development of atherosclerotic lesions. Mice heterozygous for the apolipoprotein E deficiency *(apoe⁺/)* were repeatedly challenged with systemic inoculations of the live periodontal pathogen

through intravenous inoculations (Li *et al,* 2002). Mice receiving the challenge of *Pg* showed a statistically significant increase in mean aortic lesion area compared with vehicle controls, providing direct evidence that a long-term systemic challenge with the oral pathogen *Pg* can accelerate atherosclerotic plaque progression. Although this study clearly demonstrated a link between infection by an oral bacteria and atherosclerotic lesion development, the systemic intravenous route of inoculation does not accurately reflect the natural cycle of infection of *Pg,* and therefore as stated by Li *at al.*, it does not, in fact, reproduce infections that occur in patients with periodontal disease.

2.2 Oral infection

In order to mimic the oral infection of *Pg* seen in individuals with periodontal disease, Lalla *et al* (Lalla *et al,* 2003) designed a study to assess the impact of an oral *Pg* infection on the development of atherosclerotic lesions in *apoe⁻/* mice. Rather than the intravenous route used by Li *et al,* This group chose to inoculate male *apoe ⁻/ mice* via an oral challenge of *Pg.* Mice receiving the oral challenge demonstrated evidence of a local periodontal infection as measured by an increase in alveolar bone loss, one of the characteristic traits of periodontal disease (Al-Bayaty *et al,* 2011). Infected mice also displayed evidence of generalized activation of the immune system as measured by an increase in serum immunoglobulin G (IgG) levels. Additionally, PCR analysis of the aortic tissue of infected mice found that *Pg* localized at the site of lesion development within the aorta. In this model infection with live *Pg* intensified the early stages of atherosclerosis development, with analysis revealing a 40% increase in mean lesion area in mice that received the oral challenges compared to control animals (Zhou *et al,* 2005). These results demonstrate that oral infection with this periodontal pathogen accelerates early atherosclerotic lesion development, via a route of inoculation that accurately reflects the natural cycle of *Pg* infection in individuals with periodontal disease. The findings reported in this study are significant in that they represent the first experimental evidence that entry of *Pg* through the oral cavity into the hosts system may magnify vascular inflammation and early atherosclerotic lesion formation. With a relationship established between *Pg* and atherosclerosis, the focus of research is now to identify the inflammatory genes and pathways involved in the progression from periodontal disease to atherosclerosis (Scannapieco *et al*, 2003).

To investigate the inflammatory response to *Pg,* Bodet et al, made use of a human *ex vivo* whole blood model. Whole blood from six healthy individuals was challenged with three different strains of the *Pg* bacterium. Following this challenge, enzyme-linked immunosorbant assay (ELISA) was employed to assess cytokine production. All of the *Pg* strains tested induced a significant increase in several pro-inflammatory cytokine levels, including interleukin (IL)-1β. While these studies clearly demonstrate that a relationship exists between periodontal diseases, in particular *Pg,* and atherosclerosis, they provide little or no evidence as to the mechanism involved in the progression from an oral infection to atherosclerosis (Bodet *et al,* 2006).

3. Caspase-1

Caspases are a family of cysteine proteases that carry out critical roles in mammalian apoptosis (Nicholson *et al,* 1999) and share a stringent specificity for cleaving target proteins at the peptide bond C-terminal to aspartic acid residues (Boatright *et al,* 2003). A study conducted by Kuida *et al* (Kuida *et al,* 1995) identified the enzyme caspase-1 as being responsible for processing the inactive IL-1β precursor into the mature pro-inflammatory cytokine. Using mice deficient in the caspase-1 gene, Kuida *et al* (Kuida *et al,* 1995) observed that monocytes deficient in this enzyme were not able to produce and secrete mature IL-1β. Studies conducted by Martinon *et al* (Martinon and Tschopp, 2004), Gu *et al* (Gu *et al.,* 1997) and Loppnow *et al* (Loppnow *et al.,* 1998) further investigated the role of caspase-1 in the maturation of other pro-inflammatory cytokines, and reported that in addition to the processing of IL-lβ, caspase-1 is also responsible for the activation of IL-18 (IGIF, interferon-y inducing factor).

Caspase-1 was originally identified as the enzyme responsible for the maturation of the pro-inflammatory cytokines IL-1β (Kuida *et al,* 1995) and IL-18 (Gu *et al,* 1997; Loppnow *et al,* 1998; Martinon and Tschopp, 2004). As outlined below, recently a number of laboratories have demonstrated that both IL-1β and IL-18 promote atherosclerosis in the apolipoprotein E deficient *{apoe⁻/)* mouse model. Kirii *et al* (Kirii *et al,* 2003) designed a study to examine the role of IL-1β in the development of atherosclerotic lesions using an *apoe⁻/* mouse model deficient in endogenous IL-1β. IL-1β is a pro-inflammatory cytokine that is responsible for a variety of actions, including initiation of cyclooxygenase type 2 (Lee *et al,* 2004), type 2 phospholipase A (Pascual *et al,* 2003), and inducible nitric oxide synthase (Hashimoto *et al,* 2003). In their study, it was found that there was a decrease in atherosclerotic lesion formation of approximately 30% in mice lacking IL-1β, as compared to those competent for IL-1β. These results demonstrate that a lack of IL-1β decreases the severity of atherosclerosis in *apoe⁻/* mice. Given that caspase-1 is also responsible for the activation of another proinflammatory cytokine, IL-18, another study designed to examine the role of IL-18 in the development of atherosclerosis using a mouse model that was deficient in DL-18. IL-18 plays a key role in innate immune responses through the activation of macrophages (Munder *et al,* 1998), T-helper type 1cells (Cooper et al, 1997; Decken et al, 1998), and natural killer cells (Kawakami et al, 2000). It was found that mice lacking endogenous IL-18 demonstrated approximately a 40% reduction in mean atherosclerotic lesion size as compared to control mice (Whitman et al, 2002). These preliminary results suggest a role for IL-18 in the development of atherosclerosis. The results of these studies outline a possible mechanism for *Pg* mediating atherosclerotic lesion development through the activation of caspase-1 and subsequent maturation of DL-18 and IL-1β.

4. P gingivalis promotes atherosclerosis in *vivo* via caspase-1

In previous studies a role for caspase-1 in atherosclerotic lesion development *in vivo* was elucidated by demonstrating that a deficiency in endogenous caspase-1 protected atherosclerosis susceptible male and female *apoe⁻/* mice from developing lesions.

Additionally, Lalla and colleagues (Lalla *et al,* 2003) assessed the impact of *Pg* infection on atherogenesis in male *apoE⁻/⁻* mice, revealing that oral infection with this periodontal pathogen accelerates early atherosclerosis. Studies conducted by Chi et al, Lalla et al, as well as Li et al (Chi et al., 2004;Lalla et al, 2003;Li et al, 2002) in apoe$^{-/-}$ mouse model, to study the involvement of *Pg* in lesion formation, however the attention of these studies were focused only on lesion formation in male mice. Since atherosclerosis is not a gender-specific disease, Whitman lab examines the role of caspase-1 in lesion formation as a result of *Pg* infection in both male and female atherosclerosis susceptible apoe$^{-/-}$ mice (Whitman *et al.,* 2004).

5. Conclusions

Several epidemiological studies have drawn a link between periodontal disease and atherosclerosis. *Pg* is a key pathogen in periodontal disease that has been identified in atherosclerotic lesions of both humans and mice. Previous studies have demonstrated that an oral infection with *Pg* is capable of increasing atherosclerotic lesion development in a mouse model for atherosclerosis. *Pg* has been shown to increase the expression of the pro-inflammatory cytokines IL-18 and IL-lβ, both of which have been shown to promote atherosclerosis. The enzyme responsible for the maturation of both of these cytokines into their mature forms has been identified as caspase-1. Preliminary studies conducted by the Whitman lab have demonstrated the ability of caspase-1 to promote atherosclerosis in a mouse model for the disease and the results indicating that an oral infection with *Pg* significantly enhances atherosclerotic lesion development in male apoe$^{-/-}$ mice that contain a functional caspase-1 enzyme compared to mice not receiving the oral challenge, These results outline a significant role for caspase-1 in the progression of atherosclerotic lesions in male mice mediated by an oral infection with *Pg*.

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