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Review Article

Aggregatibacter actinomycetemcomitans and Atherosclerosis

Dinh Tam Nguyen, Jack Anesi, Michelle Steicke and Yutang Wang*

Discipline of Life Sciences, School of Science, Psychology and Sport, Federation University Australia, Ballarat, Victoria, Australia

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ABSTRACT

Periodontal disease is an inflammatory condition around the teeth which affects 20-50% of the worldwide population. In periodontal disease, the bacterial plaque destroys the epithelium of the periodontal pocket and breaks the barrier that separates the tissue and the circulation, allowing oral bacteria and their endotoxins and exotoxins to enter the bloodstream. This can cause health problems, such as atherosclerosis. *Aggregatibacter actinomycetemcomitans* (Aa) is commonly found in patients with periodontitis and the number of Aa is associated with atherosclerotic lesion size in humans. This review focuses on Aa and atherosclerosis with an emphasis on the interaction of Aa with cell types involved in atherosclerosis formation.

Introduction

Periodontal disease is an inflammatory condition around the teeth including the gum, periodontal ligament and alveolar bone. In the USA, severe periodontal disease affects about 14% - 23% of Americans and 20% to 50% of the worldwide population [1-3]. In periodontal disease, the bacterial plaque destroys the epithelium of the periodontal pocket and breaks the barrier that separates the tissue and the circulation, allowing oral bacteria and their endotoxins and exotoxins to enter the bloodstream which can cause health problems [4-6]. Indeed, oral bacteria have been detected in the carotid endarterectomy [5]. The oral bacterium *Aggregatibacter actinomycetemcomitans* (Aa) is found in 40-100% of patients with early-onset and aggressive forms of periodontitis, and in 30-40% of chronic periodontitis adult patients [7]. Periodontal bacterial burden and the number of Aa are associated with atherosclerosis size in humans [8, 9]. This mini review focuses on Aa and atherosclerosis with an emphasis on the interaction of Aa with cell types involved in atherosclerosis formation.

A Brief Overview of Atherosclerosis Initiation and Progression

Atherosclerosis is an inflammatory disease in which lipoproteins accumulated in the arterial wall. It accounts for 50% of deaths in developed countries [10]. Dietary lipids are packaged into large triglyceride-rich lipoproteins called chylomicrons. These are secreted into the lymph and in the blood via the thoracic duct [11]. Very low-density lipoprotein loses its triglycerides in the peripheral tissue to develop into low-density lipoprotein (LDL) and the latter can be oxidized to its oxidized form (ox-LDL) under oxidative and inflammatory conditions. LDL and ox-LDL may accumulate in the arterial intima mainly by macrophages [12, 13]. Macrophages engulf ox-LDL, but cannot utilize these lipids, and eventually transform into lipid-laden foam cells. This is the beginning of the formation of atherosclerosis [14]. Foam cells secrete inflammatory cytokines, reactive oxygen species, and other mediators [15]. In such an environment, macrophages can die, forming the 'necrotic' core of the mature plaque. Matrix metalloproteinases, produced by macrophages, degrade extracellular matrix macromolecules that lend to the strength of the plaque's fibrous cap. Consequently, the fracture of a weakened fibrous cap permits blood to contact tissue factor in the plaque's necrotic core, ultimately triggering thrombotic plaque complications including myocardial infarction, stroke, and cardiovascular death [15-17].

Aggregatibacter actinomycetemcomitans (Aa)

Aa is a non-spore-forming, non-motile hemolytic, oxidase- and catalase-positive and gram-negative anaerobic coccobacillus [18]. Aa forms small, translucent or transparent colonies with irregular edges that

**Correspondence to: Yutang Wang, Discipline of Life Sciences, School of Science, Psychology and Sport, Federation University Australia, PO Box 663, University Drive, Mt Helen, Victoria, 3350, Australia; Tel: 61353279394; E-mail: yutang.wang@federation.edu.au*

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appear smooth, circular and convex approximately 0.5-1.0 mm in diameter [18]. Aa has different serotypes and serotypes a, b and c are dominant and comprise more than 95% of Aa [19, 20]. Aa invades and migrates through epithelial cells [21]. Aa can secrete 179 different proteins that include the endotoxin lipopolysaccharide (LPS) repeats in toxin immunostimulating factor and extracellular matrix protein adhesin A [19, 20, 22-26]. transforming growth factor-beta (TGF-β) receptor/smad2 plays an important role in the maintenance of homeostasis in the gingival epithelium [27]. Aa accelerates the expression of TGF-β receptor/smad2, which causes apoptosis of the epithelial cells, leading to the loss of the epithelial barrier [28].

The Correlation of Aa with Atherosclerosis

It has been reported that dental disease is positively correlated with an increased risk of coronary heart disease, with atherosclerosis the key underlying mechanism [7]. Poor oral hygiene, determined by the extent of dental debris and calculus, is also associated with an increased incidence of coronary heart disease [7]. For more details on the correlation between periodontal disease and atherosclerotic vascular disease, please refer to the excellent review authored by Lockhart *et al.* [29]. Aa can be found in atherosclerotic plaque of mice intravenously challenged with Aa, as well as in human atherosclerotic plaque [5, 30]. Using deconvolution micrographs, Aa has been detected in carotid atherosclerosis plaque regions [5]. The number of Aa is also associated with atherosclerosis size in humans [8].

In apolipoprotein E-deficient spontaneously hyperlipidemic mice, atherosclerotic plaque size is increased after treatment with Aa [30]. A range of pro-inflammatory cytokines in the serum of Aa-treated mice are increased and those cytokines include interleukin-6 (IL-6), IL-8, tumor necrosis factor-alpha (TNFα) and monocyte chemoattractant protein-1 (MCP-1) [30]. Toll-like receptor 2, Toll-like receptor 4, intercellular adhesion molecule 1 (ICAM-1), E-selectin, P-selectin, Ox-LDL receptor-1, heat shock protein 60, chemokine ligand 19 and 21, chemokine ligand-receptor 7, and MCP-1 expression in the aorta are significantly increased in mice challenged with Aa [30].

The inflammasome related molecules interferon-inducible protein absent in melanoma 2 (AIM2), macrophage inducible Ca^{2+}-dependent lectin receptor CLEC4E, and nucleotide-binding oligomerization domain leucine-rich repeat and pyrin domain-containing protein 3 (NLRP3) are present in the aorta [31]. AIM2 is an important component in the inflammasome complex: it can cleave the pro-inflammatory IL-1 family of cytokines into the bioactive forms of IL-1β [32]. Macrophage inducible Ca^{2+}-dependent lectin receptor CLEC4E is a C-type lectin receptor capable of recognizing a wide range of lipid species [33]. NLRP3 inflammasome is considered a link between lipid metabolism and inflammation [34]. The roles of NLRP3 inflammasome in atherosclerosis have been reviewed by Hoseini [34]. Aa promotes the production of IL-1β and IL-18 through the stimulation of NLRP3 inflammasome [35]. Oxidative modification of LDL arises from lipid peroxidation of polyunsaturated fatty acids of phospholipids within LDL particles [36]. Lipopolysaccharide from Aa is found to LDL accumulation in RAW 264.7 macrophages, forming foam cells [37].

Interaction of Aa with Various Cell Types Involved in Atherosclerosis Formation

I Cell Types Involved in the Formation of Atherosclerosis

Various cell types are involved in the pathogenesis of atherosclerosis. Dysfunction of the endothelial cells lining the lesion-prone areas of the arterial vasculature is an important contributor to the pathobiology of atherosclerosis [38]. In lesion-prone arterial regions, the actions of pro-inflammatory factors (e.g. endotoxin) and ox-LDL lead to endothelial activation [38]. Endothelial activation induces the expression of adhesion molecules and recruitment of monocytes, T-lymphocytes, B-lymphocytes, dendritic cells and mast cells to the subendothelial space [39-41]. The activated endothelial cells and other cell types will produce pro-inflammatory cytokines and reactive oxygen species within the vessel wall. This environment promotes foam cell formation and prevents high-density lipoprotein particles from participating in cholesterol efflux and removal from foam cells [41]. Ultimately, the aging foam cells die and form a necrotic lipid core, further progressing atherosclerosis [38].

II Aa and Endothelial Cells

Aa elicits a pro-atherogenic response in endothelial cells in the form of increased leukocyte adhesion, heightened production of pro-inflammatory cytokines and chemokines, and increased pro-thrombotic properties [42-44]. Human coronary artery endothelial cells stimulated with Aa increase the production of IL-6, IL-8, ICAM-1 and platelet endothelial cell adhesion molecule [45]. Increases of IL-6 and IL-8 attract monocytes to the endothelium [46, 47]. When human coronary artery endothelial cells are challenged with Aa, the expression level of ICAM-1 or platelet endothelial cell adhesion molecule 1 is significantly enhanced, but this phenomenon is not observed when cells are exposed to heat-killed Aa [45]. Leukotoxin, an Aa-produced toxin, induces apoptosis and decreases cell viability of endothelial cells [48, 49]. Treatment with leukotoxin increased levels of both ICAM-1 and vascular cell adhesion protein 1 (106), which can result in rolling, adhesion and transmigration of monocytic cells into the vessel wall [50]. When endothelial cells are treated with Aa-derived LPS, the cytokines and chemokines (G-CSF, IL-8, RANTES, ICAM-1 and IL-6) secretion increase compared to untreated control [51, 52]. However, the concentration of TNF-α, IL-7, IL-16, TNF-R1, IL-1ra and IL-1, are higher but not significant in 3D culture compared to control. The concentrations of all those soluble factors except for platelet-derived growth factor-BB are significantly higher in 3D-*Aa*-LPS than in 2D *Aa*-LPS [52].

III Aa and Macrophages

Aa has strong cytotoxic activity against macrophage human cell line U937, resulting in apoptosis [53]. Y4 strain of Aa caused 60% apoptosis in U937 cells, and this is also observed in human monocytic cell line THP-1, via the p38 MAPK pathway [53-55]. Aa is also shown to kill monocytes in minutes to a few hours [56]. In addition, exposure of human monocytes to leukotoxin increased caspase 1 up to five folds within 10 to 20 [57].

IV Aa and T Lymphocytes

Aa can stimulate T-cells to produce higher levels of pro-inflammatory cytokine IL-22 and the transcription factor master-switch gene aryl hydrocarbon receptor (AhR), which is implicated in their differentiation [58]. T helper-17 (Th17) cells are a subset of pro-inflammatory cells and are defined by their production of IL-17, IL-17A, F, and also, I IL-21 and 22. $CD4^+$ T cells, isolated from atherosclerosis coronary blood vessels, express IL-17 [59]. In addition, circulating IL-17 is shown to be associated with the progression and severity of carotid artery plaques [60]. Aa infected ApoE-deficient mice have been shown to increase significantly in splenic IL-17+ and Th17 related serum cytokines (IL-1β, IL-6, IL-17 and TGF-β), as well as Th17related gene expression **(**IL-1β, IL-6, IL-17 Receptor A, IL-21, TGF-β, and STAT 3) [31, 61, 62].

Aa infected mice also increase 3 times the relative expression of Th17 related microRNA (miR-155 and miR146b) in mouse and human atherosclerosis [63]. Mir-155 has been shown to promote the development of inflammatory Th17/Th1 cell subsets [63, 64]. Mir-146b is involved in the pathogenesis of murine viral myocarditis by regulating Th17 differentiation [60].

V Aa and Dendritic Cells

Aa can stimulate dendritic cells to produce higher levels of pro-inflammatory cytokine IL-6 and TNF-a [58]. Actinomyces spp. promote activation of dendritic cells on human monocyte dendritic cell lines by induction of CD40, CD83 and CD86 [65]. CD40 ligand and CD70 activation have been shown to be of significance in an Aa rat model of periodontal disease-mediated bone reabsorption [66]. Aa has also been proven to increase CD14+ monocytes that promote increased Th17 and IL-17 immune responses in periodontal diseases [67]. Aa serotype b has an increased ability to produce Th1 and Th17 cytokine production in dendritic cells [68]. Th1 and Th17 promote both cell-mediated immunity and inflammatory response, respectively. In the presence of Aa subtype b, higher levels of IL-1β, IL-6, IL-12, IL-23, IFN-γ and TNFα are released in comparison to other serotypes [68, 69]. Dendritic cells stimulated with serotype b Aa have an increased TLR2 dependent cytokine transcription activation pathway [69]. Th-17 cytokine production increases in Aa Apolipoprotein E-deficient mouse models, suggesting a link between Aa, dendritic cell produced Th-17 and an increase in atherosclerotic plaque production [31].

VI Aa and Mast Cells

The mast cell is a leukocyte that is a granulocyte derived from the hematopoietic stem cell, which serves to respond to pathogens and modulate innate and adaptive immune responses [70]. Aa induced the secretion of TNF-α and MCP-1 in mast cells [71, 72].

Conclusion

Oral bacterium Aa plays an important role in atherosclerosis formation and progression in people with periodontitis. Aa and its endotoxins and exotoxins can interact with endothelial cells to produce adhesion molecules, leading to inflammatory cell recruitment into the subendothelial space. Aa and its endotoxins can also promote foam cell formation and release of pro-inflammatory cytokines, which then facilitate the initiation and progression of atherosclerosis (Figure 1).

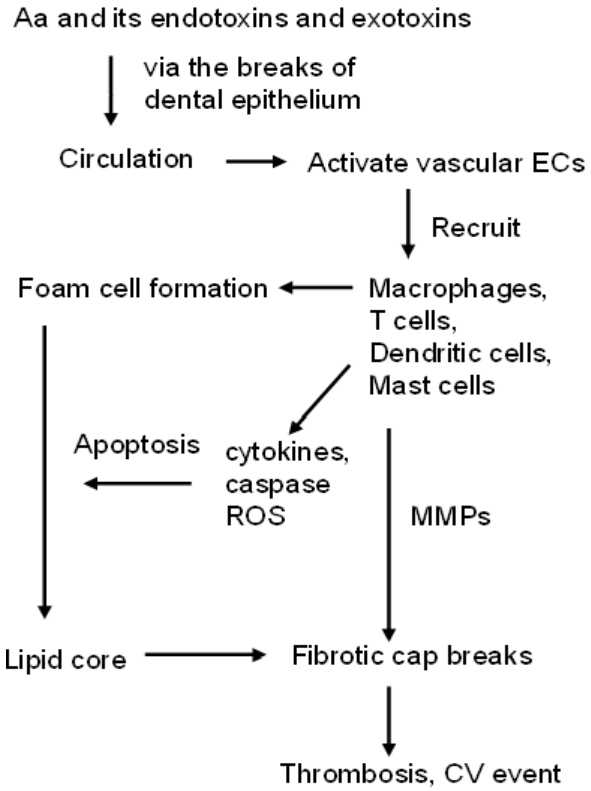

Figure 1: Aa induced atherosclerosis and cardiovascular events. Aa and its endotoxins and exotoxin exotoxins enter the circulation via breaks of the dental epithelium. They activate endothelial cells (ECs) and lead to the recruitment of inflammatory cells. Macrophage will engulf LDL and ox-LDL leading to foam cell formation. The inflammatory environment in the subendothelial space will cause apoptosis of foam cells and lead to the formation of lipid core. In addition, inflammatory cells will produce matrix metalloproteinases (MMPs) which will weaken and break the fibrotic cap. Consequently, contact of blood with tissue factor in the lipid core will lead to thrombosis and cardiovascular (CV) events such as myocardial infarction and stroke. Aa: *Aggregatibacter actinomycetemcomitans*; LDL: Low-Density Lipoprotein; ox-LDL: oxidized Low-Density Lipoprotein; ROS: Reactive Oxygen Species.

Abbreviations

Aa: *Aggregatibacter actinomycetemcomitans*
ICAM-1: Intercellular Adhesion Molecule 1
IL: Interleukin
LDL: Low-Density Lipoprotein
LPS: Lipopolysaccharide
MCP-1: Monocyte Chemoattractant Protein-1
NLRP3: Nucleotide-Binding Oligomerization Domain Leucine-Rich Repeat and Pyrin Domain-Containing Protein 3
ox-LDL: oxidized LDL
Th17: T helper-17
TNF*α*: Tumor Necrosis Factor-Alpha
TGF-β: Transforming Growth Factor-Beta

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