Heat Shock Proteins and Periodontitis – Cross-Reaction Between Bacterial and Human HSP in Periodontal Infection Linking with Cardiovascular Diseases

Tadashi Yamamoto and Takanori Eguchi

Abstract

Introduction Periodontitis is a globally prevalent chronic inflammatory disease caused by oral dysbiotic biofilm. The host immune and inflammatory responses against the biofilm play a crucial role in the initiation and progression of periodontitis. Many members of heat shock proteins (HSP) are upregulated by varieties of stresses in periodontal pathogenic bacteria, such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. HSP are immunodominant antigens in periodontitis, however, it has been unclear whether/how anti-HSP reactions involve the pathogenesis of periodontitis as well as cardiovascular diseases. In here, we aim to clarify whether/how anti-HSP reactions involve the pathogenesis of periodontitis and cardiovascular diseases.

Methods We review the current knowledge of oral bacterial and mammalian HSP and discuss immunoreaction to HSP in periodontitis.

Results Among bacterial HSP, GroEL is well investigated in auto-immune reactions because of the sequence similarity with human Hsp60. The antibody titers to

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Hsp60 and bacterial GroEL were significantly higher in the periodontitis patients and these antibodies were also detected in the gingival tissue and atherosclerotic plaques.

Conclusions The anti-HSP reactions may be involved in the pathogenesis of periodontitis as well as cardiovascular diseases. The precise mechanism of the cross-reaction between bacterial and mammalian HSP remains unclear. However, the elevated levels of the antibodies play important roles in periodontal immune and inflammatory response and may correlate the pathogenesis and the severity of periodontal and cardiovascular diseases. Further understanding of the roles of HSP might lead to a new theranostic strategy of precision medicine for systemic health linked periodontitis.

Keywords *Aggregatibacter actinomycetemcomitans* · Autoimmunity · Bacterial HSP · Cardiovascular disease · Periodontitis · *Porphyromonas gingivalis*

Abbreviations

A. actinomycetemcomitans	*Aggregatibacter actinomycetemcomitans*
CPN	chaperonin
EPF	early-pregnancy factor
HSP	heat shock protein
LDL	low-density lipoprotein
MAA-LDL	malondialdehyde-acetaldehyde-modified LDL
NEF	nucleotide exchange factor
OxLDL	oxidized LDL particles
P. gingivalis	*Porphyromonas gingivalis*
TLR	Toll-like receptor

1 Introduction

Periodontitis is a chronic inflammatory disease caused by oral polymicrobial infection. More than 700 species of oral bacteria that develop into a biofilm, called dental plaque, have been identified. The inter- and intra-individual variation in microbiota depends on the environment, genetics, age, and lifestyle of the host. These risk factors can spatiotemporally influence the microbiota composition and colonization, resulting in dysbiosis; disruption in the normal balance between the oral commensal flora and the innate immune status of periodontal tissue. The subgingival dysbiotic biofilm mediates the destruction of supporting connective tissue and alveolar bone around the teeth and triggers the periodontal inflammation, consequently leading to tooth loss. Periodontitis is one of the most prevalent infectious diseases and can affect up to 90% of the worldwide population. Although moderate periodontal inflammation and bone loss are common in older persons, severe periodontitis should not be regarded as a natural consequence of aging. High susceptibility to periodontitis, termed as aggressive periodontitis, has been reported in children and young adult patients that are characterized by rapid destruction of the periodontal

tissues in otherwise healthy individuals. [10, 18, 39, 42]. The considerable burden of massive tooth loss includes structural disorder in occlusion and temporomandibular joint, physiological dysfunction in mastication and nutrient intake, and even troubles of pronunciation, esthetics, emotion, and oral health-related quality of life. Importantly, periodontitis does not only cause tooth loss, but also adversely affects systemic health.

Over the past two decades, a new paradigm shift has occurred in periodontology with the concept "Periodontal medicine". Periodontal medicine is a collective term used to describe how periodontal infection/inflammation may affect extraoral health [5]. Periodontitis has been potentially linked to over 50 systemic diseases and conditions may increase the patients' risk particularly for cardiovascular disease including atherosclerosis, aspiration pneumonia, diabetes mellitus, adverse pregnancy outcomes, rheumatoid arthritis, and Alzheimer's disease (Fig. 1), even after

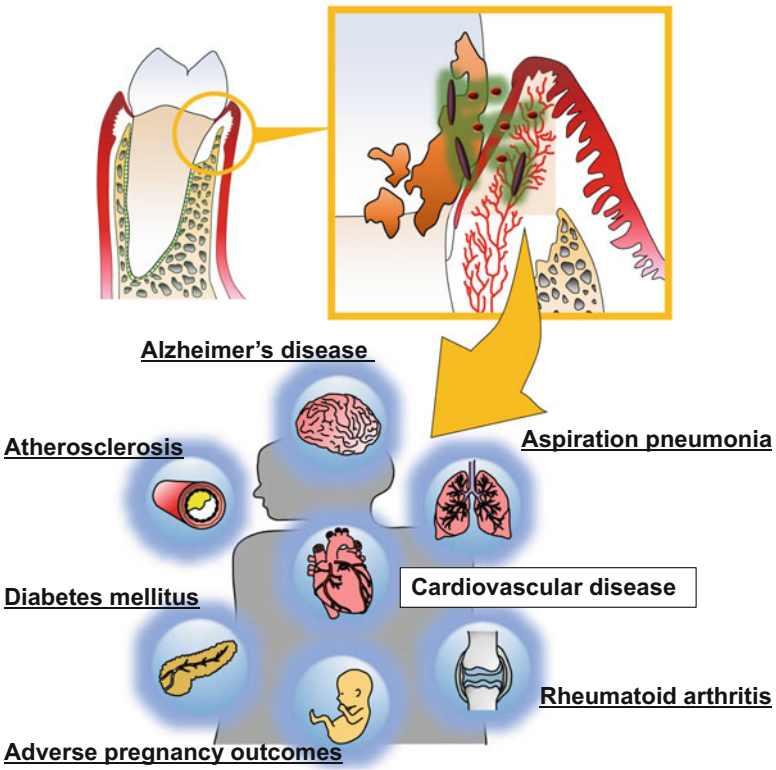

Fig. 1 Periodontal medicine – a relationship between periodontitis and systemic diseases. Periodontitis is pathologically correlated with many systemic disease states, such as cardiovascular diseases including atherosclerosis, aspiration pneumonia, diabetes mellitus, adverse pregnancy outcomes, rheumatoid arthritis, and Alzheimer's disease. Periodontal polymicrobial infection and the pro-inflammatory products circulatory spread to the whole body and adversely affect the systemic conditions. Chronic low-grade inflammations contribute to the generation of a systemic inflammatory phenotype. Epidemiological and experimental studies have suggested the most strong link between periodontitis and cardiovascular disease among such systemic diseases

adjusting for other known risk factors such as age, gender, smoking, body mass, *etc*. Although the underlying mechanisms linking these diseases are not fully understood, accumulated data have indicated that periodontitis can elicit a systemic inflammatory response, occurred during transient and recurrent bacteremia caused presumably by periodontal infections. The ulcerated epithelium of the periodontal pocket facilitates the proximity of the subgingival dysbiotic biofilm to the underlying connective tissue and alveolar bone. Periodontal inflammation by bacterial invasion and influx of immune cells leads to leakage of bacterial products, inflammatory mediators, and pathogenic oral bacteria into the blood circulation through which they are transported to distal sites. Eventually, the dissemination of bacterial spread and chronic low-grade inflammatory condition adversely affect systemic diseases (Figs. 1 and 2). More importantly, increasing shreds of evidence have suggested that periodontal treatment can mitigate the risk of systemic diseases. Periodontal treatment consists of mechanical debridement and antimicrobial chemotherapy to remove oral dysbiotic biofilms and may consequently reduce bacteremia and local and systemic inflammatory response [24].

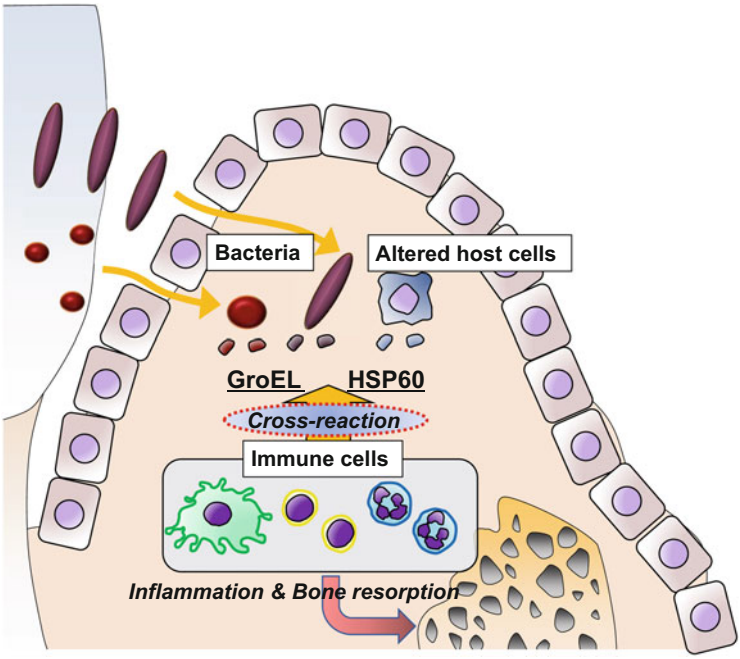

Fig. 2 Immune inflammatory response in the periodontium. The junctional epithelium of gingiva closely adheres to the tooth surface through the hemidesmosome and provides a physiological barrier to bacterial exposure. Bacteria and bacterial virulence factors disrupt the integrity of gingival epithelium, leading bacterial invasion and inflammatory infiltration of immune cells in the sub-connective tissue. Periodontal immune responses are highly cross-reactive against bacterial GroEL and HSP60 synthesized in the stressed host cell, resulting in the progression of inflammatory responses and bone resorption around the teeth

Gram-negative anaerobes and spirochetes are closely associated with periodontitis and are referred to as periodontal bacteria [47]. Although the bacterial biofilm plays a crucial role in the initiation and progression of periodontitis, the prolonged inflammation and severity of the disease mainly depend on the host immune and inflammatory responses. The immune-inflammatory response is complex and involves both innate and acquired immunity and the cytokine and inflammatory mediator networks. In the early lesion, both the host and bacteria in the periodontal biofilm release proteolytic enzymes that damage gingival tissue. They also release cytokines and chemokines that recruit neutrophils into sites of infection. If the recruited neutrophils fail to control the dysbiotic microbiota, the chronic lesion is initiated, characterized by the phagocytosis and digestion of bacteria and bacterial products by macrophages and neutrophils that can recognize pathogen *via* toll-like receptors (TLR). Complement proteins are also activated. If the early lesion persists without resolution, the advanced lesion is induced with a transition from innate immune response to the acquired immune response. The antigen-antibody reaction is a major host-protective mechanism that is processed and presented by lymphocytes, macrophages, and dendritic cells. In the inflamed periodontal tissue, macrophages, plasma cells, and T and B lymphocytes are dominant, with IgG1 and IgG3 subclasses of B lymphocytes also present. Tissue destruction is thought to be mediated by many mediators and cytokines, including prostaglandin E2 (PGE2), interleukin-1 (IL-1), IL-6 and tumor necrosis factor-alpha (TNF-α), which induce the production of matrix metalloproteinases by fibroblasts and receptor activator of nuclear factor kappa-B ligand (RANKL) resulting in collagen and extracellular matrix degradation and bone resorption. All information indicates that the tissue destruction during periodontitis is not primarily due to infectious agents, but rather the result of a persistent but not effective immune and inflammatory response. Excellent reviews of more detailed mechanisms exist elsewhere, e.g. [8, 34].

1.1 Bacterial HSP as Potent Antigens in Periodontitis

There is evidence that heat shock proteins (HSP) are immunodominant antigens derived from many periodontal bacteria [31]. HSP are families of highly conserved proteins and are abundantly found in virtually all living organisms, from bacteria to humans. HSP are commonly grouped into subfamilies based on molecular weight: small HSP, GroES-homologue proteins also known as HSP10 (~ 10 kDa), DnaJ-homologue proteins also called HSP40 (~ 40 kDa), GroEL-homologue proteins also known as HSP60 (~ 60 kDa), DnaK-homologue proteins also called HSP70 (~ 70 kDa), HptG-homologue proteins also called HSP90 (~ 90 kDa), and Clp ATP-dependent proteases also called HSP100. HSP belonging to the same subfamily share the strong amino acid sequence identity [16] (Table 1). These proteins were first described as a response to heat shock and the main role is to allow microorganisms to survive under stress conditions, but they are also expressed during tissue remodeling or wound healing and are associated with several pathological

Table 1 Bacterial HSP and corresponding human homologs

Bacterial homolog	Human homolog and alternative names	Functions
DnaK	Hsp70	(i) Ubiquitously expressed HSP
		(ii) Forms machinery important for protein folding, and help to protect cells from stress
DnaJ	Hsp40	(i) An enzyme that couples cycles of ATP binding, hydrolysis, and ADP release to cycles of sequestration and release of unfolded proteins
		(ii) The J domain interacts with Hsp70. DnaJ plays a role in regulating the ATPase activity of Hsp70
GroEL	Hsp60, Cpn60, HspD1, mitochondrial matrix protein P1, p60 lymphocyte protein	(i) A mitochondrial chaperonin, which is typically responsible for the transportation and refolding of proteins from the cytoplasm into the mitochondrial matrix
		(ii) A chaperonin to assist in folding linear amino acid chains into their respective three-dimensional structure
		(iii) Involved in diabetes, stress response, cancer, and other immunological disorders
GroES	Hsp10, Cpn10, EPF, HspE1	(i) Required for the proper folding of many proteins
		(ii) Requires the cochaperonin protein complex GroES
GrpE	HSP 20-30kD	GrpE dimer serves as the nucleotide exchange factor (NEF) for DnaK
HtpG	–	Class III HSP (molecular chaperone)

Cpn chaperonin, *EPF* early-pregnancy factor

functions in inflammatory diseases. HSP act as molecular chaperones that assist in proper protein folding and re-folding as well in the transport of damaged or toxic unfolded proteins to degradation systems [16, 54]. Periodontal bacteria are often exposed to a wide range of stresses (temperature, pH, redox potential, oxidative stress, etc.) that may affect their growth and virulence and induce a stress response. Many HSP are quickly upregulated under stressful conditions. Pre-existing immunity to HSP from various pathogens could cross-react with host natural HSP causing the autoimmune reactions. The high sequence similarity of HSP among species explains the cross-reaction possibilities between host and pathogenic HSP [21].

Periodontal immune responses against HSP also can be highly cross-reactive among bacterial and mammalian species and capable of recognizing both foreign and self-stress proteins [2, 16]. Homologs of specific stress protein families have been demonstrated to be present in many periodontal bacteria. Among them, HSP of *Porphyromonas gingivalis* (*P. gingivalis*) and *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) have been extensively studied [16]. *P. gingivalis* shows a strong association with periodontitis, although only

present at a low frequency, and is pathogenic capable of inducing dysbiosis and thereby act as a keystone pathogen [11]. *A. actinomycetemcomitans* is also a major causative bacterium by promoting bacterial invasion into the gingival tissue and elicits an excessive immune response [12]. The JP2 clone of *A actinomycetemcomitans* is considered as a pathogen of aggressive periodontitis [19]. *P. gingivalis* and *A. actinomycetemcomitans* possess several HSP and the syntheses of GroEL, GroES, DnaK, and HtpG are all up-regulated following heat stress [16] (Table 1). The GroEL homologs are known to be key molecules in autoimmune reactions because of the sequence similarity with human Hsp60 and the reactions by *P. gingivalis* GroEL may be one of the causes for the initiation of periodontitis [33]. *P. gingivalis* GroEL and *A. actinomycetemcomitans* GroEL also share a high degree of homology. Human Hsp60 but not *P. gingivalis* or *A. actinomycetemcomitans* GroEL demonstrated a stimulatory activity on the production of tumor necrosis factor-alpha (TNF-α) in THP-1 monocytic cells. Therefore, Hsp60 family proteins from different bacteria species have different proinflammatory activities to induce proinflammatory cytokines [52].

HSP family proteins lack an N-terminal hydrophobic signal sequence, characteristic of most secreted proteins, and therefore, cannot be released from cells by the conventional secretion pathways. However, HSP can be secreted into the extracellular milieu *via* secretory lysosomes or lipid vesicles including ectosomes and exosomes. Formation of the microvesicles consists of complex processes including the internalization of portions of the plasma membrane and subsequent release of microvesicles containing a variety of functional mRNA, microRNA, and intracellular proteins, including HSP [7]. *A. actinomycetemcomitans* GroEL is located on the cell surface, in surface-associated material, and on outer membrane vesicles (OMV) produced by *A. actinomycetemcomitans* meanwhile, *P. gingivalis* GroEL is not encapsulated in the OMV of *P. gingivalis*, possibly suggesting the difference in the virulence of the bacteria [16, 46]. Even so, both GroEL homologs are immunodominant antigens in humans and are presented to macrophages as foreign antigens by lymphocytes and involved in the mediation of bone resorption [22, 28, 44, 45] (Fig. 2). As the molecular chaperone functions, Hsp60 and Hsp70 have been reported to play important roles in antigen presentation and cross-presentation, activation of macrophages, lymphocytes, and dendritic cells to induce proinflammatory mediators. Therefore, it has been suggested that the HSP provides the link between innate and adaptive immune systems. In periodontitis patient's sera, high levels of circulating antibodies to periodontal pathogen and host-derived antigens, and the levels of antibodies to *P. gingivalis* correlated with IgG antibodies to Hsp60 as well as Hsp70 [6]. In addition, these innate immune responses are mediated by TLR2, TLR4, or TLR2-TLR4 heterodimers. The cytokine production activity of hsp60 was inhibited by anti-CD14 and anti-TLR4 antibodies in THP-1 monocytic cells [52]. Nevertheless, there is controversy about whether these effects are due to the contamination of recombinant HSP with pathogen-associated molecules such as lipopolysaccharide [7, 51].

1.2 HSP Increased in Periodontitis Patients

Accumulating evidence shows that Hsp60 is highly expressed in inflamed periodontal tissues in periodontitis patients. Hsp60 was highly expressed in inflamed tissue samples from periodontitis patients compared with samples from periodontally healthy individuals [2, 20, 32, 52]. Moreover, it has been shown that gingival tissues extracted from periodontitis patients contained antibodies to the *P. gingivalis* GroEL [48]. Autoimmunity with cross-reaction between human Hsp60 and *P. gingivalis* GroEL has been suggested to be a feature of periodontal disease [21] (Table 1). The antibody titers to Hsp60 and *P. gingivalis* GroEL were significantly higher in the periodontitis patients compared to a control group and those antibodies were detected in all patient-derived samples of gingival tissue extracts of periodontitis [48]. In periodontitis patients, Hsp60 or *P. gingivalis* GroEL-reactive T-cell clones and periodontitis lesion-infiltrating T cells share the same receptors, suggesting that Hsp60-specific and *P. gingivalis* GroEL cross-reactive T-cells accumulate in periodontitis lesion. Analysis of the cytokine profiles demonstrated that Hsp60-reactive T-cell clones from periodontitis patients produced significantly higher levels of gamma interferon compared with cells from healthy control, whereas *P. gingivalis* GroEL did not induce. These data suggest that periodontal infection may activate self Hsp60-reactive T cells with Th1 cytokine profile. Subsequent activation of macrophage and Th2 cytokine secretion may induce antibody production specific to bacteria as well as endogenous Hsp60 [56]. *A. actinomycetemcomitans* GroEL is also an immunodominant antigen in humans. Immunological cross-reactivity between fibronectin and *A. actinomycetemcomitans* GroEL that may lead to an autoimmune response and contribute to tissue destruction during the progression of periodontitis [57]. *A. actinomycetemcomitans* GroEL can modulate acquired and adaptive immune responses [35] although the detailed mechanism remains elusive. Sera collected from patients with periodontitis reacted strongly with *A. actinomycetemcomitans* GroEL [23], however, antisera *A. actinomycetemcomitans* GroEL highly cross-react with *E. coli* GroEL and weakly cross-react with Hsp60, suggesting that the part of serum antibody to *A. actinomycetemcomitans* GroEL could be derived from the cross-reactivity with enterobacteria *E. coli* GroEL and self-Hsp60 [49] (Table 1).

1.3 HSP in Cardiovascular Disease

Cardiovascular disease, including atherosclerosis, myocardial infarction, and stroke, is the leading cause of death worldwide, and atherosclerosis is the major underlying etiology. Atherosclerosis is generally initiated by mononuclear cell infiltration into the intima at arterial branches and curves. Infiltration precedes the accumulation of cholesterol and recruitment of macrophages and vascular smooth muscle cells and occurs when endothelial cells are stressed by traditional risk factors such as cigarette smoking, hypertension, elevated lipid levels, and diabetes mellitus. A sizable percentage of patients may not have any of the risk factors, therefore, chronic infection

and inflammation have been focused on the initiation and progression of cardiovascular events. Epidemiologic data have suggested a potential link between periodontitis and cardiovascular disease [4, 24]. Low-grade chronic systemic inflammation and increased circulating cytokines such as C-reactive protein (CRP), IL-1, IL-6, TNF-α, and PGE2, are linked to adverse cardiovascular outcomes and anti-inflammatory agents can prevent cardiovascular disease [41]. Periodontitis is associated with elevated levels of CRP and other inflammatory biomarkers and periodontal therapy can lower the serum levels of the inflammatory biomarkers and reduce the risk factors by improving endothelial function [36, 50]. Periodontal bacteria and their products are also considered to have a role either directly through the cytotoxicity or indirectly by inducing inflammation in the progression of cardiovascular disease, as atherosclerotic plaque contains viable and invasive periodontal bacteria, such as *P. gingivalis* and *A. actinomycetemcomitans* [25]. These bacteria are proposed to exert atherogenic effects by accumulating at sites of plaque development and provoke the local vascular inflammatory response [14].

Indirect interactions of periodontal pathogens in cardiovascular disease include both innate and acquired immune responses. High combined IgG response to *A. actinomycetemcomitans* and *P. gingivalis* was associated with prevalent cardiovascular disease although it is not entirely clear how the antibodies participate in the pathogenesis [38]. Another immunologic mechanism to explain the association is the antigen mimicry between GroEL and human HSP. GroEL cross-reacts with endogenous Hsp60 expressed on endothelial cells, leading to endothelial dysfunction and atherogenesis [37, 53]. Circulating levels of Hsp60 have been correlated with susceptibility to cardiovascular disease [55] whereas antibodies to Hsp60 accelerate and perpetuate the atherosclerotic process by promoting infiltration of mononuclear cells into the intima and significant correlation was observed between anti-Hsp60 antibody and high morbidity and mortality of atherosclerosis [54]. Hsp60 was selectively found in arterial endothelial cells and lymphocytes in atherosclerotic lesions rather than non-atherosclerotic areas, while GroEL and periodontal bacteria were detected within intimal cells in carotid endarterectomy specimens, suggesting that Hsp60 and GroEL closely associate with inflammatory infiltrate of activated T cells and macrophages [13]. Epidemiologic data have reported significant positive correlations between anti-*P. gingivalis* GroEL levels and anti-Hsp60 in cardiovascular patient's sera, as *P. gingivalis* GroEL proteins are highly homologous to Hsp60 [27]. Previous studies have identified T-cell immune responses specific to *P. gingivalis* GroEL in patients suffering from atherosclerosis and periodontitis [9]. Cross-reactive antibodies and T-cells between Hsp60 and *P. gingivalis* GroEL have been detected in the peripheral blood of patients with atherosclerosis as well as in the atherosclerotic plaques themselves [15] (Table 1). The autoimmune concept reinforced the concept that the development of tolerance against Hsp60 and its peptides could be useful for the prevention of atherosclerosis. Several studies demonstrated the protective effect by using recombinant Hsp60 or Hsp peptides vaccination. Sublingual vaccination with recombinant GroEL is also effective for the prevention of *P. gingivalis*-associated atherosclerosis in an experimental mice model. However, clinical studies by using antibiotic therapy have been investigated gave equivocal results for effects on atherosclerosis [17, 54].

Lipid-modification is a principal therapy for the prevention of atherosclerosis. There are several available and emerging therapies for lowering low-density lipoprotein (LDL) cholesterol [40]. In the atherosclerotic intima of the artery wall, LDL particles go through oxidative modification which creates oxidized LDL particles (OxLDL) and subsequently malondialdehyde-acetaldehyde-modified LDL (MAA-LDL) particles are created. The exact role of antibodies to OxLDL in atherosclerosis is not fully understood. Since IgM antibodies to OxLDL inhibit the cholesterol uptake of macrophages through scavenger receptors, IgM antibodies to OxLDL are considered to have protective properties, whereas IgG antibodies to OxLDL are more heterogeneous but mainly considered pro-atherogenic [26]. The MAA adducts have potent immunogenicity and dose-dependent direct cellular toxicity [3]. Increased levels of MAA adducts were detected in cardiovascular disease and periodontitis lesion and salivary IgA antibodies to MAA-LDL cross-react with *P. gingivalis*, Rgp44 (gingipain A hemagglutinin domain of *P. gingivalis*) and *A. actinomycetemcomitans* in patients with cardiovascular disease [1]. Moreover, *A. actinomycetemcomitans* GroEL cross-reacts with Hsp60 and MAA-LDL, which could be a potential mechanism in the progression of cardiovascular disease [26] (Fig. 3). Therefore, controlling immunoreaction to LDL and HSP in periodontitis is beneficial for the prevention and management of cardiovascular diseases.

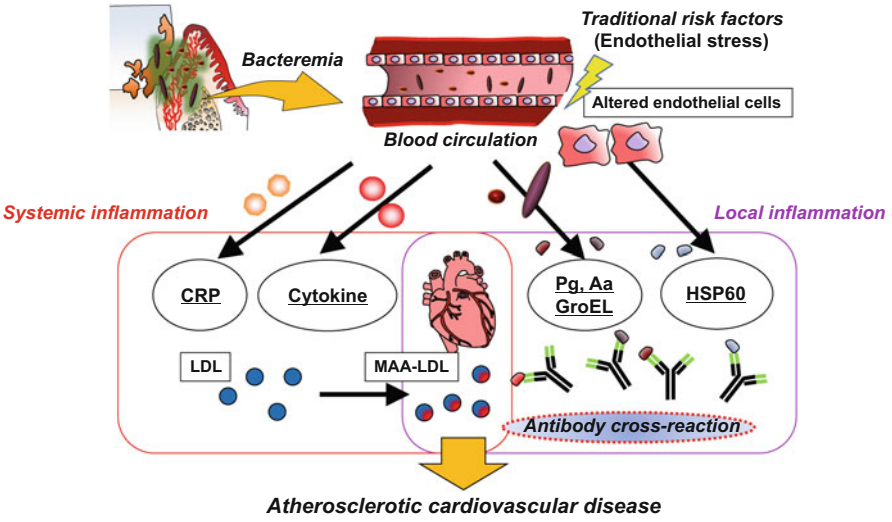

Fig. 3 Autoimmune concept in periodontitis-associated atherosclerosis. Periodontal bacteremia can spread into coronary lesions through blood circulation. Endothelial cells in the lesion are stressed by traditional risk factors such as cigarette smoking, hypertension, elevated lipid levels, diabetes mellitus, and consequently synthesized HSP60. Low-grade chronic systemic inflammation and increased circulating cytokines such as CRP, are linked to the modification of LDL to generate malondialdehyde-acetaldehyde-modified LDL (MAA-LDL). GroEL, HSP60, and MAA-LDL released by *P. gingivalis* and *A. actinomycetemcomitans* are highly immunogenic and cross-reactive each other. Eventually, the dissemination of bacterial spread and chronic inflammatory conditions adversely enhances atherosclerotic cardiovascular disease

As mentioned so far, many molecular and immunologic mechanisms have been proposed, the exact mechanism linking between periodontitis and cardiovascular disease is yet to be fully known. However, it has become increasingly evident that periodontal bacterial HSP generate antibodies that can cross-react with human HSP and these antibodies activate inflammatory cytokine production, as well as monocyte and endothelial cell activation in the atherogenic process [43]. Although much evidence of epidemiological linkage between two diseases has been reported, the significance of the correlation depends on the types of study design. According to an American Heart Association statement, the association is independent of known confounders [30]. The most recent Cochrane review described that there is no reliable evidence for primary and secondary prevention of cardiovascular disease by periodontal treatment. Further trials are needed to reach conclusions about whether periodontal treatment can help prevent the occurrence or recurrence of cardiovascular disease [29].

2 Conclusions

The precise mechanism of the cross-reaction between bacterial and mammalian HSP remains unclear. However, the elevated levels of the antibodies play important roles in periodontal immune and inflammatory response and may correlate the pathogenesis and the severity of periodontal and cardiovascular diseases. Longitudinal intervention and pathogenic mechanism studies are required. Further understanding of the roles of HSP and LDL might lead to a new theranostic strategy of precision medicine for systemic health linked periodontitis. Importantly, reducing the systemic risk associated with periodontitis requires new biomarkers and diagnostic tools that can link the pathological condition of the diseases and improve diagnostic capability, and establishing clinical guidelines for the treatment.

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Ethical Approval for Studies Involving Animals This article does not contain any studies with animals performed by any of the authors.

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