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Salivary IgA to MAA-LDL and Oral Pathogens Are Linked to Coronary Disease

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Abstract

A large body of literature has established the link between periodontal disease and cardiovascular disease. Oxidized low-density lipoproteins (OxLDLs) have a crucial role in atherosclerosis progression through initiation of immunological response. Monoclonal IgM antibodies to malondialdehyde-modified low-density lipoprotein (MDA-LDL) and to malondialdehyde acetaldehyde–modified low-density lipoprotein (MAA-LDL) have been shown to cross-react with the key virulence factors of periodontal pathogens *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. We have previously shown that salivary IgA antibodies to MAA-LDL cross-react with *P. gingivalis* in healthy humans. In this study, we aim to assess whether oral mucosal immune response represented by salivary IgA to MAA-LDL and oral pathogens is associated with coronary artery disease (CAD). Also, the molecular mimicry through antibody cross-reaction between salivary IgA to MAA-LDL and oral pathogens was evaluated. The study subjects consisted of 451 patients who underwent a coronary angiography with no CAD (n = 133), stable CAD (n = 169), and acute coronary syndrome (ACS, n = 149). Elevated salivary IgA antibody levels to MAA-LDL, Rgp44 (gingipain A hemagglutinin domain of *P. gingivalis*), and Aa-HSP60 (heat shock protein 60 of *A. actinomycetemcomitans*) were discovered in stable-CAD and ACS patients when compared to no-CAD patients. In a multinomial regression model adjusted for known cardiovascular risk factors, stable CAD and ACS were associated with IgA to MAA-LDL (P = 0.016, P = 0.043), Rgp44 (P = 0.012, P = 0.004), Aa-HSP60 (P = 0.032, P = 0.030), *Tannerella forsythia* (P = 0.002, P = 0.004), *Porphyromonas endodontalis* (P = 0.016, P = 0.020), *Prevotella intermedia* (P = 0.038, P = 0.005), and with total IgA antibody concentration (P = 0.002, P = 0.016). Salivary IgA to MAA-LDL showed cross-reactivity with the oral pathogens tested in the study patients. The study highlights an association between salivary IgA to MAA-LDL and atherosclerosis. However, whether salivary IgA to MAA-LDL and the related oral humoral responses play a causal role in the development in the CAD should be elucidated in the future.

Keywords: lipids, atherosclerosis, bacterial virulence, biomarkers, mucosal immunity, periodontal disease(s)/periodontitis

Introduction

Oral mucosal immunity plays a crucial role in maintaining homeostasis in oral microbiome communities, and one way of achieving this is by secreting immunoglobulins (Brandtzaeg 2013). Dysbiosis in the oral microbiome leads to the development of diseases such as periodontitis (Kinane et al. 2017). The association of atherosclerosis with oral pathogens and periodontal disease has been established (Beck et al. 1996). Atherosclerosis is a chronic inflammatory disease; the disease starts by accumulation of low-density lipoproteins (LDLs) beneath the intima of the artery wall (Gistera and Hansson 2017). Subsequently, retained lipoproteins go through oxidative modification, leading to the formation of oxidized LDL (OxLDL) (Gistera and Hansson 2017). OxLDL plays a key role in the inflammation of arterial wall and atherogenesis. Oxidized LDLs are also present in saliva fluid (De Giuseppe et al. 2015), and recently, Bright et al. (2018) reported that malondialdehyde acetaldehyde (MAA) adducts are increased in gingivitis and periodontitis lesions.

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A supplemental appendix to this article is available online.

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Oxidized epitopes include oxidized phospholipids (OxPLs) and malondialdehyde (MDA)–modified amino groups (Binder et al. 2016). They are presented not only in OxLDL but also in several biological structures, such as apoptotic cells and damaged proteins, representing danger (or damage)–associated molecular patterns (DAMPs). Oxidized epitopes are recognized by pattern recognition receptors and the proteins of the innate immune system, leading to removal and prevention of inflammatory effect (Binder et al. 2016). MAA epitopes are the final product of reaction between MDA and acetaldehyde. It has been proposed that the MAA adducts have potent immunogenicity, unique stability, and dose-dependent direct cellular toxicity among aldehyde adducts (Antoniak et al. 2015). Previously, we have shown that newborn babies possess natural IgM antibodies to MAA epitopes, and those antibodies may regulate apoptotic cell clearance during fetal development (Wang et al. 2013). In addition, we and others have proposed that MAA adducts contribute to pathogenic mechanisms in which classical risk factors of cardiovascular disease, such as hyperlipidemia and diabetes, may initiate inflammation and lead to development of atherosclerosis (Veneskoski et al. 2011; Antoniak et al. 2015). Anderson and colleagues have shown that coronary artery disease (CAD) patients possess higher levels of plasma IgA and IgG antibodies to MAA-LDL than controls (Anderson et al. 2014). In contrast, IgM antibodies to MDA-LDL have an inverse association with carotid atherosclerosis (Karvonen et al. 2003).

Porphyromonas gingivalis is a gram-negative bacterium that is considered crucial in periodontitis development due to its central role in the orchestration of microbial dysbiosis (Hajishengallis 2015). Several studies have shown high levels of serum IgG antibodies to *P. gingivalis* in atherosclerotic vascular diseases (Pietiäinen et al. 2018). Animal models infected by oral *P. gingivalis* have also shown enhanced development of atherosclerosis compared to controls (Lalla et al. 2003). Previously, our group has shown that the immunization of atherosclerotic animal models with heat-killed *P. gingivalis* increased plasma IgM to MDA-LDL, and monoclonal IgM antibodies to MDA-LDL recognized *P. gingivalis* virulence factor gingipain (Rgp44) as an antigen (Turunen et al. 2012). The data reveal the existence of cross-reactive epitopes or molecular mimicry between MDA and the virulence factor. We have also shown that antibodies to a virulence factor of another key microbe in periodontitis, *Aggregatibacter actinomycetemcomitans* heat shock protein 60 (Aa-HSP60), cross-react with MAA-LDL (Wang et al. 2013). HSP60 is the most relevant and well-studied member of the heat shock protein family with regard to autoimmunity and development of atherosclerosis due to molecular mimicry between bacterial HSP60 and human HSP60 (Kilic and Mandal 2012).

We have recently shown that healthy subjects have salivary IgA antibodies to oxidized LDL (Akhi et al. 2017). In the present study, we investigated whether levels of salivary antibodies to MAA-LDL and common oral bacteria are associated with coronary artery disease. Also, the molecular mimicry through antibodies' cross-reaction between salivary IgA to MAA-LDL and oral pathogens was assessed.

Materials and Methods

Study Subjects and Examinations

The current study consists of 451 patients originating from the Corogene prospective study, which was constituted by 5,295 subjects who were assigned to coronary angiography in the region of southern Finland (Vaara et al. 2012). A subset (about 10%) of participants in the Corogene study was randomly invited to an extensive oral examination (Buhlin et al. 2011). Exclusion criteria were previous heart transplantation, low hemoglobin, or blood transfusion during the same hospitalization period (Vaara et al. 2012). The medical history was obtained from hospital records. Patients were asked to complete a questionnaire reviewing their smoking status and oral hygiene habits, and they were classified according to medications (Vaara et al. 2012). Patients were considered smokers if they smoked at the time or had quit smoking less than 6 mo ago. Coronary artery disease was diagnosed according to coronary artery angiography, symptoms, and clinical examination. "No CAD" was categorized if coronary arteries had nonsignificant stenosis (≤50%) and "stable CAD" if the stenosis was >50% in at least 1 coronary artery. Acute coronary syndrome (ACS) was defined as >50% of stenosis in at least 1 coronary artery and an episode of typical ischemic chest pain (Vaara et al. 2012). The oral examination was performed by 2 calibrated periodontists and previously published (Buhlin et al. 2011). Periodontal disease was categorized as "healthy" = no alveolar bone loss (ABL) and bleeding on probing (BOP) <25%, "gingivitis" = no ABL and BOP >25%, and periodontitis = mild to severe ABL. Saliva samples were collected and stored at −80°C. Pooled subgingival bacterial samples were collected from the deepest pathological periodontal pocket (≥4 mm) of each dentate quadrant and analyzed by checkerboard DNA-DNA hybridization (Mäntylä et al. 2013). This investigation conformed to STROBE guidelines for investigational studies.

Preparation of Antigens and Chemiluminescence Immunoassay

LDL (density 1.019–1.063 g/mL) isolation from human plasma was carried out by sequential density ultracentrifugation (Hörkkö et al. 1999). Oxidization of freshly isolated LDLs with 5 μM copper sulfate (CuOx-LDL) was done as previously described (Hörkkö et al. 1999). Malondialdehyde acetaldehyde modification of LDL (MAA-LDL) was performed by mixing 20% acetaldehyde, LDL, and freshly prepared MDA. The pH was calibrated to 4.8 and the mixture was incubated at +37°C for 2 h. Excessive aldehydes were extracted by overnight dialysis against 0.27 mM EDTA in phosphate-buffered saline (PBS) at +4°C. The bacterial antigens are listed in the supplemental appendix. The antigens from *P. gingivalis* and *A. actinomycetemcomitans* were a mixture of various serotypes. Bacteria were cultured on fastidious anaerobe agar supplemented with 5% to 10% blood. Heat-killed bacteria were prepared by incubation at 60°C for 1 h in PBS. Recombinant

P. gingivalis virulence factor, gingipain Rgp44, was prepared as previously described (Turunen et al. 2012). The 60-kDa Aa-HSP60 was prepared as previously described (Wang et al. 2016). Levels of salivary IgA and IgG antibodies to oxidized LDL and bacterial epitopes were determined by chemiluminescence immunoassay as previously described (Karvonen et al. 2003). Please see detailed description of the methods and the intra-assay coefficient of variation (CV) in the materials and methods section of the supplemental appendix.

Statistical Analysis

All statistical analyses were performed using SPSS statistics software (SPSS, Inc.). Parameters with skewed distributions were logarithmically transformed and geometric means were calculated. The differences between characteristics of the CAD groups were analyzed with the Kruskal-Wallis test and Pearson χ^2 test. The difference in the antibody levels between the groups was analyzed by 1-way analysis of variance (ANOVA) and post hoc Dunnett test. The association of salivary IgA and IgG antibodies to studied antigens with stable CAD and ACS was analyzed by a multinomial logistic regression model adjusted by clinical confounders: age, gender, smoking, diabetes (yes/no), hypertension (yes/no), dyslipidemia (yes/no), total DNA probe, and body mass index (BMI). For analysis of the competition assay, Wilcoxon test pairwise statistics for 2 related samples was used. *P* value below 0.05 was considered significant. The correlation of salivary IgA antibody to MAA-LDL and oral pathogens was examined with bivariate Pearson analysis. The association of salivary IgA and IgG antibody to OxLDL with the oral pathogen level in the periodontal pocket was analyzed by the Kruskal-Wallis test. The error bars represent the standard error (SE). The subgingival pathogen DNA levels are shown as medians with interquartile ranges (IQRs). The numbers of missing variables in statistical tests are presented in Appendix Table 1.

Results

Baseline Characteristics

The basic characteristics and cardiovascular risk factors are presented in the Table. The subjects' age varied between 33 and 82 years, with a mean of 63.3 years, and age variation between groups was significant ($P < 0.001$). On average, the population was overweight (mean BMI, 27.8 kg/m^2); 67% of the male participants were overweight. Most of the patients had medications for cardiometabolic disorders and the variation between groups was statistically significant: hypertension ($P = 0.03$), dyslipidemia ($P < 0.001$), and diabetes mellitus ($P = 0.001$). A significant variation in *A. actinomycetemcomitans* ($P = 0.046$) subgingival levels was observed between patient groups. Increased level of saliva total IgA was detected in patients with stable CAD and ACS ($P = 0.001$) in comparison to no-CAD patients, whereas total salivary IgG concentrations did not differ between the groups.

Increased Level of Salivary IgA to MAA-LDL, Rgp44, and Aa-HSP60 in Patients with Stable CAD and ACS

The mean levels of salivary IgA and IgG antibodies to oxidized LDL epitopes, MAA-LDL, and CuOx-LDL are presented in Figure 1. Patients with stable CAD ($P = 0.003$) and ACS ($P = 0.044$) had significantly higher levels of IgA antibodies to MAA-LDL than no-CAD patients. The levels of IgA and IgG antibodies to CuOx-LDL did not differ between the groups (Fig. 1A, B). Patients with stable CAD and ACS had significantly higher IgA antibody levels of saliva to *P. gingivalis* gingipain Rgp44 ($P = 0.017$ and $P = 0.005$, respectively) and to *A. actinomycetemcomitans* HSP60 ($P = 0.014$ and $P = 0.018$, respectively) (Fig. 1A) in comparison with no-CAD patients. Salivary IgG levels to Rgp44 and Aa-HSP60 did not differ between the groups (Fig. 1B).

Salivary IgA Antibodies to Oral Pathogens in Patients with Angiography-Verified CAD

The salivary IgA and IgG antibody levels to periodontal pathogens are presented in Figure 2. Patients with stable CAD had significantly higher salivary IgA antibody levels to *P. gingivalis* ($P = 0.039$), *Porphyromonas intermedia* ($P = 0.019$), *Porphyromonas endodontalis* ($P = 0.001$), *Tannerella forsythia* ($P = 0.002$), and *A. actinomycetemcomitans* ($P = 0.014$) than the no-CAD group (Fig. 2A). Patients with ACS had increased salivary IgA levels to *P. intermedia* ($P = 0.005$), *P. endodontalis* ($P = 0.007$), and *T. forsythia* ($P = 0.003$) (Fig. 2A) in comparison with the no-CAD group. Salivary IgG levels to periodontal pathogens did not differ between the groups (Fig. 2B).

Salivary IgA Antibodies to MAA-LDL Epitopes and Periodontal Pathogens Associated with CAD

The association of salivary IgA antibody levels with the CAD status was analyzed by a multinomial regression model (Fig. 3). After adjusting for established cardiovascular risk factors, stable CAD associated significantly with IgA to MAA-LDL ($P = 0.016$), Rgp44 ($P = 0.012$), Aa-HSP60 ($P = 0.032$), *T. forsythia* ($P = 0.002$), *P. endodontalis* ($P = 0.016$), *P. intermedia* ($P = 0.038$), and total IgA ($P = 0.002$) antibody levels (Fig. 3A). ACS associated with IgA levels to MAA-LDL ($P = 0.043$), Rgp44 ($P = 0.004$), Aa-HSP60 ($P = 0.030$), *T. forsythia* ($P = 0.004$), *P. endodontalis* ($P = 0.020$), *P. intermedia* ($P = 0.005$), and total IgA ($P = 0.016$) antibody levels (Fig. 3B). These findings were independent of periodontal disease (data not shown).

Salivary IgA Antibodies to OxLDL and Periodontal Pathogens

Appendix Figure 1 shows the correlation of salivary IgA antibody to MAA-LDL with IgA antibody to Rgp44 ($r = 0.628$,

Table. Baseline Clinical Characteristics of Subjects According to Cardiovascular Groups.

	No CAD (n = 133; 29.5%)	Stable CAD (n = 169; 37.5%)	ACS (n = 149; 33.0%)	P Value[a]
		Mean (SD)		
Age, y	61.0 (8.8)	65.8 (8.0)	62.6 (9.9)	<**0.001**
BMI, kg/m^2	27.5 (5.2)	27.8 (4.6)	28.0 (5.2)	0.612
Saliva IgA, µg/mL	323.3 (149.6)	391.4 (164.1)	371.0 (151.0)	**0.001**
Saliva IgG, µg/mL	46.6 (15.7)	49.6 (17.8)	49.2 (19.5)	0.319
		Median (IQR)		
Total DNA probe[b]	62.65 (109.74)	73.16 (105.63)	63.75 (103.05)	0.284
Porphyromonas gingivalis[b]	0.53 (3.33)	0.58 (4.27)	0.58 (3.46)	0.893
Prevotella intermedia[b]	0.72 (2.23)	0.79 (2.4)	0.79 (2.66)	0.637
Porphyromonas endodontalis[b]	0.31 (2.09)	0.26 (1.80)	0.24 (2.05)	0.723
Tannerella forsythia[b]	4.63 (13.90)	6.04 (20.63)	4.85 (21.76)	0.607
Aggregatibacter actinomycetemcomitans[b]	0.98 (4.65)	2.13 (5.31)	1.14 (3.83)	**0.046**
		n (%)[c]		P Value[d]
Gender (men)	64 (14.2)	129 (28.6)	108 (23.9)	<**0.001**
Hypertension	75 (16.7)	119 (26.4)	92 (20.4)	**0.030**
Dyslipidemia	96 (21.4)	158 (35.2)	108 (24.1)	<**0.001**
Diabetes mellitus	19 (4.3)	52 (11.6)	29 (6.5)	**0.001**
Stenosed arteries				
0	133 (29.5)	0	0	<**0.001**
1	0	43 (9.53)	71 (15.7)	<**0.001**
2	0	51 (11.3)	39 (8.6)	<**0.001**
3	0	75 (16.6)	39 (8.6)	<**0.001**
Periodontal disease				
Healthy	16 (3.5)	12 (2.7)	13 (2.9)	0.463
Gingivitis	26 (5.8)	14 (3.1)	21 (4.7)	**0.022**
Periodontitis	85 (18.8)	129 (28.6)	105 (23.3)	**0.008**
Edentate	5 (1.1)	12 (2.7)	10 (2.2)	0.431
Smoking				
No	72 (16.0)	76 (16.9)	64 (14.2)	0.142
Former	42 (9.3)	73 (16.2)	67 (14.9)	**0.044**
Current	19 (4.2)	19 (4.2)	18 (4.0)	0.730

Statistically significant P values ($P < 0.05$) are bolded. ACS, acute coronary syndrome; BMI, body mass index; CAD, coronary artery disease; IQR, interquartile range.
[a]Kruskal-Wallis test.
[b]Unit, count × 10^5.
[c]Percentages were calculated from the total population (n = 451).
[d]Pearson χ^2 test.

$P < 0.001$), *A. actinomycetemcomitans* ($r = 0.481$, $P < 0.001$), Aa-HSP60 ($r = 0.697$, $P < 0.001$), *P. endodontalis* ($r = 0.648$, $P < 0.001$), *P. intermedia* ($r = 0.580$, $P < 0.001$), *T. forsythia* ($r = 0.735$, $P < 0.001$), and *P. gingivalis* ($r = 0.476$, $P < 0.001$). Appendix Table 2 shows the association of subgingival levels of bacteria, *A. actinomycetemcomitans* with the salivary IgA to Cuox-LDL ($P = 0.016$), IgA to MAA-LDL ($P = 0.001$), and IgG to MAA-LDL ($P = 0.001$). Also, subgingival levels of *P. endodontalis* associated with salivary IgG to CuOx-LDL ($P = 0.033$) and MAA-LDL ($P = 0.003$).

Salivary IgA Binding Specificity to MAA-LDL and Oral Pathogens

The competitive binding of salivary IgA antibodies to MAA-LDL with oral pathogens and their virulence factors was investigated (Fig. 4). The IgA binding to MAA-LDL in the presence and absence of competitors was significantly different ($P < 0.001$). *P. gingivalis*, Rgp44, Aa-HSP60, and *A. actinomycetemcomitans* showed a different extent of competitive binding with salivary IgA to MAA-LDL (Fig. 4). The virulence factors competed more strongly than the whole bacteria with respect to salivary IgA binding to MAA-LDL.

Discussion

In this study, we showed that salivary IgA antibodies to MAA-LDL and periodontal pathogens were associated with angiographically verified coronary artery disease. In addition, salivary IgA to MAA-LDL showed cross-reactivity with the oral bacteria *P. gingivalis* and *A. actinomycetemcomitans* and their respective virulence factors. Molecular mimicry between

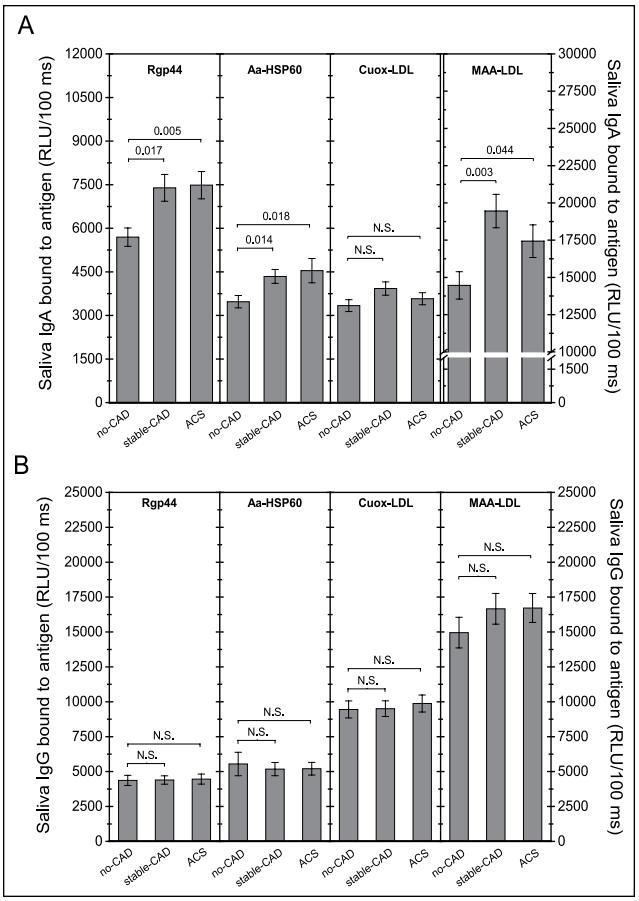

Figure 1. Antibody levels of salivary IgA (**A**) and IgG (**B**) according to cardiovascular status. The differences in antibody levels of stable coronary artery disease (CAD) and acute coronary syndrome (ACS) groups with the no-CAD group were analyzed by 1-way analysis of variance using the post hoc Dunnett test. Due to skewed data distributions, statistical tests were performed with logarithm-transformed values. The error bar represents standard error of the mean (SEM). Aa-HSP60, *Aggregatibacter actinomycetemcomitans* heat shock protein 60; CuOx-LDL, copper-oxidized low-density lipoprotein; MAA-LDL, malondialdehyde acetaldehyde–modified low-density lipoprotein; NS, not significant with $P < 0.05$; Rgp44, *Porphyromonas gingivalis* A hemagglutinin domain; RLU, relative light unit.

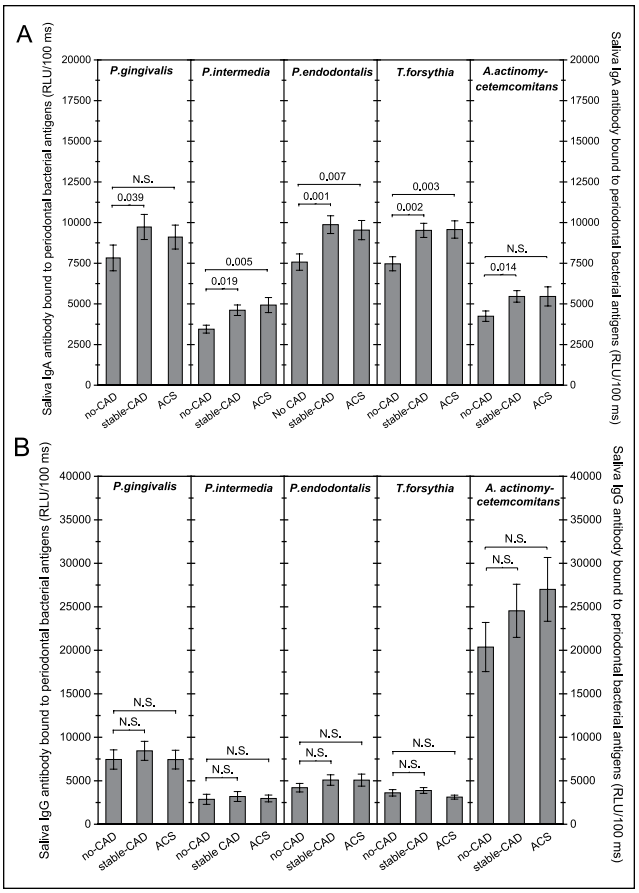

Figure 2. Antibody levels of salivary IgA (**A**) and IgG (**B**) to periodontal pathogens according to coronary artery disease (CAD) status. The differences in antibody levels of stable CAD and acute coronary syndrome (ACS) groups with the no-CAD group were analyzed by 1-way analysis of variance post hoc Dunnett test. Due to data skewed distributions, statistical tests were performed with logarithm-transformed values. The error bar represents standard error. NS, not significant with $P < 0.05$; RLU, relative light unit.

2 structurally similar epitopes may have a role in the course of atherosclerosis by activation of cross-reactive immune response (Tsiantoulas et al. 2014), and the results of the current study reveal an association between oral humoral response and coronary artery disease.

IgA is an arch of humoral immunity. In the mucosal area, the dimeric IgA are secreted by plasma cells and provide defense against pathogenic and commensal bacteria (Pabst 2012). In serum, monomeric IgA antibodies are produced by plasma cells in the bone marrow, B1 cells, and marginal zone B cells (Leong and Ding 2014). Recently, we have shown that the levels of salivary IgA and IgG antibodies to OxLDL epitopes do not correlate with the levels in plasma (Akhi et al. 2017). Limited information is available about the origin and the role of IgA antibodies to OxLDL. Our previous data have suggested that plasma IgA to OxLDL is linked to glucose metabolism, and a high level of IgA antibodies could be an independent risk factor for type 2 diabetes mellitus (Sämpi et al. 2010). We have recently reported that plasma IgA antibodies to phosphocholine (PCho) and the *Streptococcus pneumoniae* cell wall polysaccharide predict independently long-term risk of cardiovascular events (Kankaanpää et al. 2018). Furthermore, saliva from healthy human subjects has been shown to contain IgA and IgG antibodies to MAA-LDL that cross-react with the periodontal pathogen *P. gingivalis* (Akhi et al. 2017). However, the possible role of salivary IgA to MAA-LDL in atherogenesis remains elusive. In this study, a multinomial logistic regression model was built by adjusting with major cardiovascular risk factors. Stable CAD and ACS were found to be associated with a highly increased cross-reactive salivary IgA antibody to MAA-LDL compared to no-CAD controls, implying that salivary IgA to MAA-LDL has a role in proatherogenic processes.

Witztum and colleagues (Miller et al. 2011) have discussed how oxidized epitopes act as DAMPs and are subsequently recognized by the pattern recognition receptor (PRR). In studies of endogenous antigens considered in atherosclerosis, most attention has been paid to HSP60 and OxLDL epitopes. HSP60 shares high homology with mycobacterial HSP65 and has been expressed in endothelial cells in response to a high-cholesterol diet (Miller et al. 2011). This finding suggests that the original immune response against the microbial HSP65 antigen has developed a cross-reactive immune response against endothelial HSP60 (Tsiantoulas et al. 2014). Monoclonal antibodies are antibodies specific for an epitope. Our group has previously shown that a mouse natural monoclonal IgM antibody to MAA-LDL cross-reacts with Aa-HSP60 (Wang et al. 2016). Cross-reactivity has also been documented between MAA-LDL and Rgp44 (Kyrklund et al. 2018), a key etiologic agent of periodontitis (Hajishengallis 2015). This study shows salivary IgA antibody to MAA-LDL correlates and cross-reacts with periodontal pathogens, Rgp44, and Aa-HSP60. Also, salivary IgA to Rgp44 and Aa-HSP60 cross-reacted more specifically and had a stronger correlation with MAA-LDL than the whole bacteria of *P. gingivalis* and *A. actinomycetemcomitans*. Furthermore, stable CAD and ACS were found to be associated with a cross-reactive IgA antibody to Rgp44 and Aa-HSP60, but the association was nonsignificant for IgA antibodies to *P. gingivalis* and *A. actinomycetemcomitans*. The exact mechanism behind this phenomenon is not known. Obtained results provide further evidence that MAA epitopes mimic the structure of oral pathogens and their virulence factors, suggesting that the virulence factors, rather than the whole bacteria, may be directly involved in the humoral immune responses to oxidation-specific epitopes. This mechanism may have a crucial role in the initiation and progression of atherosclerosis.

Previously, animal studies have shown that OxLDL or heat-killed *P. gingivalis* immunization has an atheroprotective effect on mice models of atherosclerosis (Turunen et al. 2012; Binder et al. 2016). On the other hand, live *P. gingivalis* immunization enhanced atherosclerosis development (Lalla et al. 2003). Formerly, we showed that LDLR$^{-/-}$ mice immunized with MDA-LDL possessed reduced aortic lipid depositions after a challenge with live *P. gingivalis* compared with mice receiving only a *P. gingivalis* challenge (Turunen et al. 2015). In the present study, we have shown the cross-reaction of salivary IgA to MAA-LDL and *P. gingivalis*. It can be speculated that cross-reactivity of salivary IgA to OxLDL and *P. gingivalis* may contribute to an atheroprotective effect of OxLDL and heat-killed *P. gingivalis* immunization through epitopes' molecular mimicry.

A number of seroepidemiological studies have demonstrated that antibody response against bacterial biomarkers of periodontitis is associated with subclinical atherosclerosis, prevalent CAD, and incident CVD events (Pietiäinen et al. 2018). Recently, we showed in present population that combined serum IgA and IgG antibody levels to *A. actinomycetemcomitans, P. gingivalis, P. endodontalis, P. intermedia, T. forsythia, Campylobacter rectus*, and *Fusobacterium nucleatum* were associated with ACS, while the corresponding subgingival bacterial levels were not (Liljestrand et al. 2018). We showed in this study that salivary IgA antibodies to *T. forsythia, P. endodontalis*, and *P. intermedia* were associated with stable CAD and ACS after adjusting for atherosclerosis risk factors. Previously, more attention has been paid to the oral bacteria *P. gingivalis* and *A. actinomycetemcomitans* in researching the association between periodontitis and atherosclerosis. Our current findings indicate the involvement of other oral pathogens in coronary artery disease. Further investigations are needed to clarify the role played by those oral pathogenic bacteria in atherogenesis.

Janket and colleagues (2015) have discussed the role of oral infections in the development of metainflammation-associated diseases. Coronary artery diseases are a secondary complication of diabetes, and patients with poorly controlled diabetes

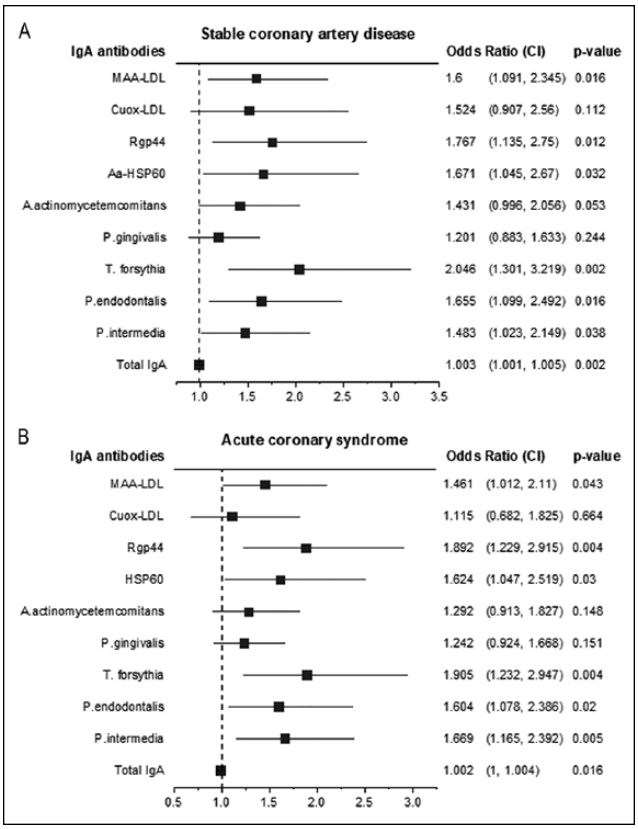

Figure 3. Levels of salivary IgA antibodies to different antigens in stable coronary artery disease (CAD) (**A**) and acute coronary syndrome (**B**) were analyzed by multinomial logistic regression. Salivary IgA levels to different antigens in no CAD were used as a reference. Model was adjusted with age, gender, smoking (never/ex/current), body mass index, diabetes, dyslipidemia, hypertension, and total DNA probe. All data except "Total IgA" were logarithm transformed due to skewed distributions, and respectively, odds ratio represents the difference in logarithmic scale. Aa-HSP60, *Aggregatibacter actinomycetemcomitans* heat shock protein 60; CI, confidence interval; CuOx-LDL, copper sulfate–low-density lipoprotein; MAA-LDL, malondialdehyde acetaldehyde–modified low-density lipoprotein; Rgp44, *Porphyromonas gingivalis* A hemagglutinin domain.

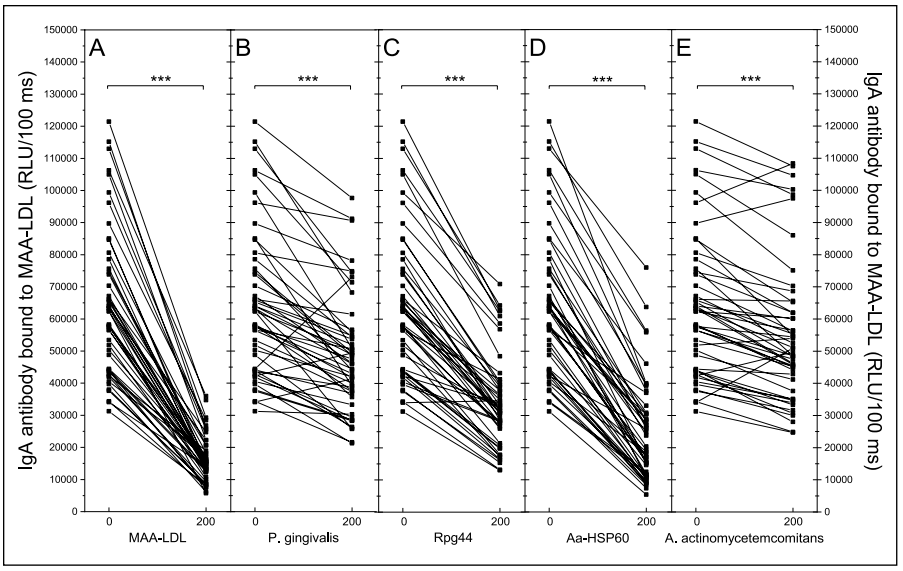

Figure 4. Comparative binding specificity of salivary IgA antibody to malondialdehyde acetaldehyde–modified low-density lipoprotein (MAA-LDL) in the absence (0 µg/mL) and presence (200 µg/mL) of soluble competitors. A total of 50 patients were chosen for the competitive immunoassay. Periodontal pathogens and their virulence factors were as competitors: (**A**) MAA-LDL, (**B**) *Porphyromonas gingivalis*, (**C**) *P. gingivalis* A hemagglutinin domain (Rgp44), (**D**) *Aggregatibacter actinomycetemcomitans* heat shock protein 60 (Aa-HSP60), and (**E**) *A. actinomycetemcomitans*. For all panels, MAA-LDL was used as a solid-phase antigen (5 µg/mL). The difference between groups was analyzed with the Wilcoxon test for 2 related samples. ***$P < 0.001$.

show an increased incidence of chronic periodontitis (Lalla and Papapanou 2011). Formerly we have shown that plasma IgA antibody levels to MAA-LDL are associated with inflammatory mediators, obesity, and type 2 diabetes (Vehkala et al. 2013). Currently, we do not know whether IgA to MAA-LDL plays a functional role in atherosclerosis or diabetes. Future studies should address these questions.

Strengths of the study include a relatively large population size ($n = 451$) and participants' verified health data. The main limitations of the current study are the cross-sectional setup and patient recruitment on the basis of an existing cohort. Other limitations of the current study are an aged population and mostly male participants. In the current study, to determine whether the groups were significantly different or if the differences were just due to random variation, we used an inferential error bar (Cumming et al. 2007).

Taken together, our study showed an increased level of salivary IgA to MAA-LDL, Rgp44, Aa-HSP60, and several oral pathogens in patients with stable CAD and ACS. Salivary IgA antibody to MAA-LDL was associated significantly with coronary artery disease and cross-reaction with Rgp44, Aa-HSP60, and periodontal pathogens. Oral mucosal immunity is constantly challenged by exogenous and endogenous antigens. This study highlights the role that mucosal humoral immune response may have on atherosclerosis development.

Author Contributions

R. Akhi, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; C. Wang, A.E. Nissinen, J. Kankaanpää, contributed to data interpretation, critically revised the manuscript; R. Bloigu, contributed to data analysis, critically revised the manuscript; S. Paju, P. Mäntylä, K. Buhlin, J. Sinisalo, contributed to data acquisition, critically revised the manuscript; P.J. Pussinen, S. Hörkkö, contributed to conception, design, data analysis, and interpretation, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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