Association of Systemic Oxidative Stress with Suppressed Serum IgG to Commensal Oral Biofilm and Modulation by Periodontal Infection

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Abstract

To assess the impact of systemic oxidative stress on humoral immune responses, we examined the relation between levels of serum 8-isoprostane and serum IgG antibodies against 17 microorganisms in the commensal oral biofilm among the ARIC population of community-dwelling adults ($n = 4,717$). Bivariately, serum 8-isoprostane was associated with age, race/center, education, smoking, serum triglycerides, and the extent of periodontal disease severity. Total IgG antibody directed to the oral biofilm was significantly associated with race/center, hypertension, triglycerides, periodontal disease severity, plaque, and serum 8-isoprostane. In multivariate models, the highest quartile of increased 8-isoprostane displayed marked reductions (44%) in biofilm IgG antibody in contrast to small increases in total IgG antibody level for the highest quartiles of oral bacterial burden or periodontal disease severity (19 and 12%, respectively; $p < 0.0001$). Increased 8-isoprostane was associated with decreased total IgG antibody ($p < 0.0001$) in subjects with or without extensive periodontal disease and/or biofilm and with suppression of IgG responses across the entire biofilm composition. Increased systemic oxidative stress is associated with a generalized decrease of serum IgG antibody responses to the oral biofilm. Levels of oral microbial burden, periodontitis severity, and smoking are, by comparison, minor modifiers of serum IgG responses to the commensal oral biofilm. *Antioxid. Redox Signal.* 11, 2973–2983.

Introduction

OXIDATIVE STRESS is an important physiologic modifier of immune (48) and inflammatory mechanisms (36). Mediators of oxidative stress or "redox signaling" or both have been shown to regulate receptor (11) and transcription factor signaling (48) and to induce NF-κB (31) and kinase-dependent signaling pathways (50), thereby inducing the expression of key cytokines (26) and inflammatory mediators. Oxidative stress can shift the balance of Th1 and Th2 cytokine profiles in *in vitro* (52) and *in vivo* model systems (47 and 53), typically toward a Th2 cytokine profile, suggesting that oxidative stress might modify humoral immune responses to commensal and pathogenic microorganisms. Clinical conditions associated with increased oxidative stress in humans [*e.g.*, smoking (56), diabetes (23), periodontitis (12), and aging (42)] have been associated with impaired immune function (44). Smoking, a recognized risk factor for periodontitis (7), has been associated with depressed levels of total serum IgG2 (55), which is a Th1-dependent IgG antibody subtype, and with decreased serum levels of antibody for selected oral

bacteria (60). Diabetes has been associated with changes in T-cell populations and decreased IgG antibody responses to oral bacteria (20). Aging has been associated with a decreased antibody response to vaccination (21). In some reports, therapies designed to reduce oxidative stress have been related to improved outcomes in viral infections and improved cell-mediated immune function in animal models and human clinical trials (10, 26, 36, 43). Thus, it is of interest to determine the association of systemic levels of oxidative stress with serum levels of IgG antibody to commensal and pathogenic microorganisms within a clinical population.

Mucosal surfaces retain a complex and diverse community of microorganisms that exist as a biofilm lining the oral cavity, the gut, the respiratory, and the reproductive tracts. Many of these organisms are commensal but can cause disease when a breach in mucosal integrity occurs. The humoral antibody response (*e.g.*, serum IgG) to organisms within the mucosal environment is generally of low concentration as a minor immunoglobulin component of mucosal secretions, but it can play an important role when a loss of mucosal epithelial integrity occurs in conditions such as gingivitis, periodontitis,

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ulcerative colitis, cystic fibrosis, and other conditions that cause inflammation or ulceration, or both, of mucosal surfaces. Most of these clinical conditions tend to be associated with increases in serum antibody titers to specific organisms within the biofilm, presumably as a consequence of bacterial presentation *via* tissue invasion or penetration of the organism, or components such as LPS. These systemic antibodies presumably also have a role in clearing bacteremias (1, 3) associated with systemic exposure to the oral microbes, a possible risk factor for systemic diseases (5, 6).

It was our general hypothesis that systemic oxidative stress, as measured by increasing serum levels of 8-isoprostane, would be a significant negative modifier of the serum-antibody responses to mucosal biofilm microorganisms. Specifically, we hypothesized that increased oxidative stress would be associated with suppression of the systemic IgG antibody responses to the indigenous oral biofilm microorganisms. To test this hypothesis, we examined the relations between serum levels of 8-isoprostane and serum levels of IgG antibodies against a panel of 17 microorganisms indigenous to the oral subgingival biofilm in a large sample of community-dwelling adults, controlling for potential modifiers of serum IgG titers and oxidative stress.

Materials and Methods

Subjects

The Atherosclerosis Risk in Communities (ARIC) study (27) is a prospective investigation supported by the National Heart Lung and Blood Institute (NHLBI) of community dwellers to characterize the natural history of atherosclerosis and of clinical cardiovascular disease in four U.S. communities (Jackson, Mississippi; Washington County, Maryland; suburban Minneapolis, Minnesota; and Forsyth County, North Carolina). The Jackson cohort comprised entirely African-Americans. A sample of 15,792 community-dwelling residents aged 45–64 years at baseline took part in an evaluation of cardiovascular risk factors and their sequelae. The Dental ARIC, an ancillary study funded by the National Institute of Dental and Craniofacial Research (NIDCR), was conducted during ARIC visit 4 in 1996 through 1998 and is cross-sectional in design. The Dental ARIC consisted of an oral examination, collection of serum, and interviews. Persons requiring antibiotic prophylaxis for periodontal probing were excluded. The Dental ARIC study was conducted among a diverse population of middle-aged adults and includes measures of a serum marker of oxidative stress (8-isoprostane), measures of the levels of a panel of serum antibodies to oral bacteria, and clinical measures of total oral microbial load (*i.e.*, plaque) and extent of periodontal disease. No exclusion criteria were used except for total edentulism and the need for antibiotic prophylaxis for the dental examination. Also, data were collected about factors known to contribute to oxidative stress (*e.g.*, smoking and diabetes). The protocols for this clinical investigation were reviewed and approved by the Institutional Review Boards of the respective institutions.

Exposure variables

Clinical measures included probing depth (PD) and cementoenamel-junction measures relative to the gingival margin (CEJ) on six sites for all teeth. Clinical attachment level (CAL) was calculated from the sum of PD and CEJ scores. Oral plaque was measured by using the Plaque Index (PI) (40). Loe Gingival Index and Bleeding on Probing were also measured. Periodontal examiners at the ARIC centers were calibrated to a standard examiner, and the range for weighted kappas was 0.86 to 0.94. Intraclass coefficients ranged from 0.87 to 0.95. Percentage of agreement within 1 mm ranged from 88.9 to 94.9%. We chose to focus our analysis by using probing depths as a measure of periodontal status, and plaque, as a measure of microbial exposure. Among the clinical signs, PD was the single best predictor of serum IgG antibody levels. To create quartiles of extent scores, participants were ranked on the percentage of sites with PD 5 mm or more or percentage of sites with PI scores of 1 or more. Participants in the top quartile of number of sites with PD of 5 mm or more or PI scores of 1 or more were considered "High." The participants in the remaining three quartiles of either PD >5 mm or PI were considered "Low."

Serum 8-isoprostane

Direct-8-iso-Prostaglandin $F_{2\alpha}$ (D-8-iso $PGF_{2\alpha}$) is a stable end product of both specific inflammatory enzymatic pathways and nonspecific mechanisms and reflects total lipid peroxidation, representing an excellent *in vivo* marker for oxidative stress (56). The D-8-iso $PGF_{2\alpha}$ assay is based on the competition between sample 8-iso-$PGF_{2\alpha}$ and a fixed amount of alkaline phosphatase (AP)-labeled 8-iso-$PGF_{2\alpha}$ for sites on a rabbit polyclonal anti-8-iso-$PGF_{2\alpha}$ (Assay Designs, Ann Arbor, MI). During the incubation, the polyclonal antibody becomes bound to the goat anti-rabbit antibody coated onto the microplate. After a wash to remove excess conjugate and unbound sample, a substrate solution is added to the wells to determine the bound enzyme activity. Color development is stopped, and absorbance read at 405 nm. The intensity of the color is proportional to the amount of AP-$PGF_{2\alpha}$ bound to the well, which is inversely proportional to the concentration of total 8-iso-$PGF_{2\alpha}$ originally present in the sample. Standard curves ranged from 100 ng/ml to 32 pg/ml, and serum samples were diluted 1:5 to yield values within the working range. Deviations of standard duplicates ranged from 1.0 to 18.8%, with a mean deviation of 5.9%. Sensitivity limit of detection was estimated by the manufacturer as 103.2 pg/ml for the 2-h incubation format. Participants were ranked on the serum concentration of D-8-iso $PGF_{2\alpha}$. With this ranking, participants in the top quartile of concentration of D-8-iso $PGF_{2\alpha}$ were considered "High." The participants in the remaining three quartiles of concentration of D-8-iso $PGF_{2\alpha}$ were considered "Low."

Outcome variables

The main outcome variables were serum IgG antibody levels of 17 selected periodontal organisms [*Porphyromonas gingivalis; Prevotella intermedia; Prevotella nigrescens; Tannerella forsythensis; Treponema denticola; Fusobacterium nucleatum; Aggregatibacter actinomycetemcomitans; Campylobacter rectus; Eikenella corrodens; Parvimonas micra; Veillonella parvula; Capnocytophaga ochracea; Seleomonas noxia; Actinomyces viscosus; Streptococcus intermedius; Streptococcus sanguis;* and *Streptococcus oralis*]. Serum samples collected as part of the ARIC examination were divided into aliquots at each examination

site, frozen at -80°C, transported on dry ice to our laboratory, and stored in aliquots at -80°C. Samples were assayed for IgG antibody levels directed against the aforementioned 17 periodontal organisms by using the checkerboard immunoblotting technique described by Sakellari *et al.* (57). This assay permits rapid quantification of serum antibody levels against a wide range of bacterial species in multiple serum samples simultaneously. In brief, bacterial antigens from each species and protein A from *Staphylococcus aureus* (Sigma, St. Louis, MO) were deposited in parallel lanes on nitrocellulose membranes (Hybond ECL; Amersham, Arlington Heights, IL), by using a Miniblotter device (Immunetics, Cambridge, MA). Whole bacterial suspensions, sonicated for 10 s and adjusted at an optical density of 1.0 (wavelength, 600 nm) were used as bacterial antigens. Subjects' serum samples in 1:500 dilution, as well as serial dilutions of human IgG immunoglobulin (100, 200, and 400 ng/ml; Sigma), were deposited in lanes perpendicular to the antigen lanes and incubated at room temperature for 1 h. After washing to remove nonbound antibody, the membrane was incubated with goat anti-human IgG peroxidase-conjugated antibody (Fab fragment; Boehringer Mannheim, now Roche, Indianapolis, IN) diluted 1:60,000 for 1 h. Positive reactions were detected with enhanced chemiluminescence, by using the ECL Western blotting detection reagents (ECL Western, Amersham Life Sciences) and the Lumi-Imager workstation (Boehringer-Mannheim). Antibody concentrations were estimated by comparing the signal intensity of unknown samples (subject sera) with those generated by the known concentrations of human IgG, captured on the same membrane, by using the LumiAnalyst software. The detection limit of the assay is ~ 20 ng/ml.

Periodontal organisms selected were representatives from clusters of organisms reported to be associated with periodontal infection and health by Socransky *et al.* (59). Subjects with periodontitis are known to have elevated serum antibody levels to periodontal organisms involved with the active infection. For example, patients with *P. gingivalis*–associated periodontitis characteristically display high serum IgG antibody titers to cell-wall LPS from this organism (17). Summation scores for total titers were made by summing IgG concentrations for each of the organisms assayed.

Covariates

Participants were defined as never smokers, former smokers, or current smokers by interview. The former and current categories were further divided into light or heavy smokers, with light smokers reporting more than none but <20 pack-years of smoking, and heavy smokers reporting ≥20 pack-years of smoking. This scheme resulted in a five-level categorization of smoking that simultaneously takes into account both temporality and magnitude of smoking status as an exposure. Education was divided into basic (<12 years), intermediate (12–16 years), or advanced (17–21 years), and was included to control for socioeconomic status. Age in years at visit 4 was included, and a variable representing race/ethnicity (African-American or White) and ARIC center was designed to control for the ethnic, regional, and examiner differences in the ARIC cohort. Persons who were not African-American or White and the few African-Americans in the Maryland and Minneapolis centers were excluded from analysis because of small numbers.

Participants fasted for 12 h before the clinical examination, and blood was collected for plasma lipids including high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC), and triglycerides, and for serum glucose. Diabetes mellitus was defined as fasting serum glucose of ≥126 mg/dl, 200 mg/dl if nonfasting, or pharmacologic treatment for diabetes, or being told by a doctor one has diabetes or sugar in the blood (self-reported).

Statistical analysis

Statistical analyses and data management were performed by using SAS (4); statistical significance was set at $p \leq 0.05$, and the unit of analysis was the person. Frequency distributions, means, empiric distribution functions, and standard errors were determined to describe the data. When distributions were skewed, log transformations were applied. Bivariate relations were investigated by using t tests for continuous variables and Cochran Mantel-Haenszel χ^2 statistics and odds ratios and 95% confidence intervals (CIs) for differences between categoric variables. Multivariable modeling was performed by using SAS Proc GLM to calculate least-squared means, adjusting for other study variables. Potential confounders were specified *a priori*, based on the literature as being associated with either exposure or outcomes. We explored the effect of infections other than periodontal disease (*e.g.*, sinus, bronchitis, kidney, and pneumonia) and potential modifiers of oxidative stress (*e.g.*, arthritis). We included in the analyses only those effect modifiers or confounders that influence the association by ≥5%, whether or not they were significant main effects.

Results

To identify factors related to serum concentrations of 8-isoprostane and total oral biofilm IgG level, pairwise analyses were conducted for relevant clinical subject-level variables (Table 1). Serum concentrations of 8-isoprostane were significantly higher among older subjects (older than 65 years) and showed a trend for increased levels among female subjects ($p = 0.07$). Serum 8-isoprostane levels also varied significantly by race/center, education, smoking, serum triglycerides, and extent of probing depth. Serum 8-isoprostane levels decreased nonsignificantly with increased plaque. Examining the relation between smoking history and serum 8-isoprostane concentrations (Table 1) shows directional differences, in that current smokers have lower 8-isoprostane concentrations than former smokers, heavy smokers have lower 8-isoprostane levels than light smokers, and current heavy smokers have lower concentrations than do nonsmokers; however, never-smokers have higher 8-isoprostane levels than do current heavy smokers. Mean serum level of total IgG antibody directed to the oral biofilm was significantly associated with race/center, hypertension, triglycerides, extent of probing depth, plaque, and serum 8-isoprostane. The association between total IgG and BMI demonstrated a trend and approached statistical significance ($p = 0.06$). A greater oral bacterial burden was associated with a significant but modest increase (*i.e.*, 19% increase) in the level of total IgG, as was more-severe periodontal disease (*i.e.*, extent of PD, ≥5 mm; 12% increase). By comparison, the impact of oxidative stress on the IgG level was marked with a 44% reduction in titer, comparing the highest quartile of 8-isoprostane with the lowest quartile. This suggests that the

TABLE 1. ASSOCIATION OF SUBJECT CHARACTERISTICS WITH SERUM 8-ISOPROSTANE
AND TOTAL ORAL BIOFILM IgG ANTIBODY LEVELS

			Median log 8-isoprostane		Mean total biofilm IgG antibody	
Group		N	Median log 8-isoprostane (ng/ml) (interquartile range)	p Value	Mean total biofilm IgG level (ng/ml) (SE)	p Value
Age	<65 yr	3,039	3.66 (1.23–5.75)		1,203.7 (26.7)	
	65+ yr	1,678	3.93 (2.45–6.00)	0.0060	1,190.0 (35.8)	0.76
Sex	Female	2,643	3.83 (1.23–6.00)		1,168.8 (29.1)	
	Male	2,074	3.67 (1.23–5.75)	0.0710	1,236.7 (31.5)	0.11
Race/field center	Jackson Co	689	3.14 (2.56–4.42)		1,533.2 (55.0)	
	NC, blacks	117	3.79 (1.23–5.38)		1,788.9 (134.1)	
	NC, whites	1,338	3.59 (2.62–5.67)		1,121.8 (39.4)	
	Wash Co	1,339	3.48 (1.23–5.59)		1,075.7 (40.1)	
	Minnesota	1,234	4.71 (2.78–6.00)	<0.0001	1,168.4 (41.9)	<0.0001
Years of education	<11 yr	631	3.50 (2.15–0.30)		1,252.2 (58.4)	
	12–16 yr	2,065	3.78 (1.23–6.00)		1,185.6 (32.3)	
	>16 yr	2,017	3.83 (1.58–6.00)	0.0476	1,196.4 (32.7)	0.60
Income ($)	<25 K	1,112	3.72 (2.11–5.73)		1,222.2 (43.6)	
	25 K ≤ 50 K	1,669	3.82 (1.23–5.81)		1,211.2 (35.5)	
	50 K+	1,759	3.79 (1.23–6.00)	0.6532	1,153.9 (34.8)	0.37
Smoking	Never	2,216	3.74 (1.23–5.77)		1,201.5 (31.3)	
	Former light	997	3.99 (2.18–6.00)		1,198.2 (46.7)	
	Former heavy	787	3.86 (2.52–6.00)		1,216.8 (52.1)	
	Current light	110	3.82 (2.69–6.00)		1,219.9 (139.5)	
	Current heavy	421	3.57 (1.23–5.69)	0.0461	1,089.4 (71.4)	0.66
BMI	<25	1,256	3.59 (1.23–5.71)		1,121.3 (41.4)	
	25 ≤ 30	1,918	3.76 (1.72–6.00)		1,194.4 (33.5)	
	30 ≤ 35	1,017	3.88 (2.06–6.00)		1,237.6 (45.9)	
	35+	518	3.74 (2.61–5.73)	0.1528	1,314.2 (64.4)	0.06
Diabetes	No	4,038	3.74 (1.23–5.98)		1,193.7 (23.0)	
	Yes	666	3.82 (2.54–5.86)	0.4516	1,216.2 (57.6)	0.71
Hypertension	No	3,108	3.73 (1.23–5.81)		1,158.5 (25.1)	
	Yes	1,585	3.80 (2.27–6.00)	0.5102	1,283.8 (40.1)	0.006
LDL	Low	2,897	3.78 (1.23–6.00)		1,210.2 (26.9)	
	High	1,818	3.72 (1.23–5.79)	0.4087	1,178.8 (35.1)	0.48
HDL	Low	3,882	3.73 (1.23–5.75)		1,243.6 (48.6)	
	High	835	3.94 (2.57–6.00)	0.2344	1,189.1 (23.8)	0.33
Triglycerides	Low	2,715	3.50 (1.23–5.41)		1,257.3 (29.8)	
	High	2,002	4.16 (2.66–6.00)	<0.0001	1,119.5 (30.0)	0.001
Extent probing depth	Low PD	3,521	3.67 (1.23–6.00)		1,167.3 (23.7)	
	High PD	1,149	3.88 (1.84–5.74)	0.0382	1,312.4 (48.5)	0.004
Plaque	Low PI	3,346	3.81 (1.23–6.00)		1,159.3 (24.8)	
	High PI	1,118	3.66 (2.55–5.24)	0.0973	1,377.8 (48.5)	<0.0001
Serum 8-isoprostane	1	NA			1,540.9 (41.9)	
Quartiles	2	NA			1,290.9 (41.7)	
	3	NA			1,080.2 (41.9)	
	4	NA			864.3 (43.1)	<0.0001

Extent probing depth, the subject quartile rank for number of sites per subject with at least 5 mm of periodontal probing depth; low PD, extent PD <5 mm Q1–Q3; high PD, extent PD >5 mm Q4; plaque, the subject quartile rank in whole-mouth score for the Loe-Silness plaque index, PI; low plq, extent PI Q1–Q3; high Plq, extent PI Q4.

For multileveled variables, the p value reflects the Cochran Mantel-Haenszel χ^2 test.

oxidative-stress status of the subject has a greater influence on the serum IgG titer than does either the level of oral bacteria present or the presence of periodontal disease, as shown by the increased extent of probing depths of 5 mm or greater, which would reflect a greater oral surface area adjacent to the biofilm.

The influence of high oral bacterial burden on serum levels of IgG antibodies and the modification by oxidative stress are shown in multivariate models in Table 2. This table demonstrates that for each plaque group shown in columns Low PI and High PI, the effect of oxidative stress significantly attenuates the IgG antibody titer with significant differences between IgG titers defined by the quartiles of 8-isoprostane ($p < 0.0001$). However, the effects of increased bacterial load on titer are statistically significant only for the lowest quartile

TABLE 2. ADJUSTED[a] MEAN TOTAL IgG BY EXTENT PLAQUE INDEX STRATIFIED BY QUARTILE OF SERUM 8-ISOPROSTANE

Serum quartiles of 8-isoprostane[b] (n)	Mean total IgG (ng/ml) (SE)		
	Mean (std err)		
	Low PI	High PI	p>|t|[a]
1 (1,019)	1,515.2 (51.3)	1,806.8 (98.1)	0.001
2 (1,048)	1,311.3 (52.6)	1,305.2 (89.5)	0.95
3 (1,033)	1,043.9 (52.9)	1,157.0 (88.5)	0.29
4 (980)	845.6 (51.8)	906.6 (102.9)	0.60
All (4,080)	1,184.7 (27.2)	1,289.2 (53.1)	0.10

[a]Adjusted for race/center (five-level), gender, BMI, age, smoking (five-level), diabetes, hypertension, education (three-level), income (three-level), probing depth, and serum LDL, HDL, and triglycerides.
[b]8-Isoprostane effect on IgG significant for both Low-PI and High-PI groups at $p < 0.0001$.

of oxidative stress (Q1; $p = 0.001$). As a result, an overall nonsignificant increase in IgG antibody level ($p = 0.10$) was noted comparing Low PI with High PI. A similar trend is evident in Table 3, which explores the effect of oxidative stress on the association between antibody titer and the extent of periodontal disease severity. Again for each periodontal disease severity group shown in columns Low PD and High PD, the effect of oxidative stress increasing Q1–4 significantly attenuates the IgG antibody titer ($p < 0.0001$). In this case, for subjects within the lowest quartile of oxidative stress (Q1), as well as in Q3, more-extensive periodontal disease is associated with a statistically significant increase in the total IgG to the oral biofilm (21% with $p = 0.002$ and 19% with $p = 0.04$, respectively). The increase in IgG in the group with more severe periodontal disease is significant overall ($p = 0.003$) in contrast to the case with plaque.

Table 4 presents similar patterns when considering subjects who are in the highest quartile for both probing depths and plaque levels, in that IgG levels decline with increasing 8-isoprostane level for each probing depth/plaque group, and an overall effect is seen according to probing depth/plaque group, independent of 8-isoprostane level ($p = 0.0007$). It also

TABLE 3. ADJUSTED[a] MEAN TOTAL IgG BY EXTENT OF PERIODONTAL DISEASE (USING EXTENT PD 5 MM) STRATIFIED BY QUARTILE OF SERUM 8-ISOPROSTANE

Serum quartiles of 8-isoprostane[b] (n)	Mean total IgG (ng/ml) (SE)		
	Mean (std err)		
	Low PD	High PD	p>|t|[a]
1 (1,019)	1,475.1 (49.1)	1,791.5 (88.3)	0.002
2 (1,048)	1,270.9 (49.1)	1,337.3 (89.9)	0.51
3 (1,033)	1,005.1 (50.4)	1,201.1 (80.7)	0.04
4 (980)	852.2 (51.0)	923.7 (90.3)	0.49
All (4,080)	1,169.3 (25.7)	1,335.8 (47.2)	0.003

[a]Adjusted for race/center, gender, BMI, age, smoking, diabetes, hypertension, education, income, plaque, and serum LDL, HDL, and triglycerides.
[b]8-Isoprostane effect on IgG significant for both High PD and Low PD groups at $p < 0.0001$.

TABLE 4. ADJUSTED[a] MEAN TOTAL IgG BY BOTH EXTENT OF PERIODONTAL DISEASE (USING EXTENT PD 5 MM) AND BY EXTENT PLAQUE INDEX STRATIFIED BY QUARTILE OF SERUM 8-ISOPROSTANE

Serum quartiles of 8-isoprostane[b] (n)	Mean total IgG (ng/ml)		
	Mean (std err)		
	Low PD + low PI	High PD + high PI	p>|t|[a]
1 (1,019)	1,458.1 (58.2)	2,309.6 (185.6)	0.002
2 (1,048)	1,297.4 (58.5)	1,310.6 (149.4)	0.93
3 (1,033)	993.5 (60.2)	1,259.8 (134.3)	0.08
4 (980)	849.3 (58.6)	970.8 (177.5)	0.19
All (4,080)	1,147.0 (30.7)	1,465.4 (84.9)	0.0007

[a]Adjusted for race/center, gender, BMI, age, smoking, diabetes, hypertension, education, income, plaque, and serum LDL, HDL, and triglycerides.
[b]8-Isoprostane effect on IgG significant for both High PD and Low PD groups at $p < 0.0001$.

is interesting to note that the highest IgG levels appear among subjects with both probing depths and plaque in the highest quartile who have the lowest oxidative stress levels ($p = 0.002$). Collectively, these data indicate that the increases in the oxidative stress level of the subjects from the lowest quartile of serum 8-isoprostane (referent group) serve to decrease strongly the total IgG response to the commensal oral biofilm relative to the lesser impact of the biofilm load and the presence of periodontal disease, both of which tend to increase the total IgG titer. However, not all organisms within the plaque are invasive pathogens associated with periodontal disease and systemic exposure, and the specificity of IgG response is explored in Table 5.

The level of total IgG stratified by quartile of 8-isoprostane is shown adjusted for race/center, age, gender, BMI, smoking, education, income, diabetes, hypertension, HDLc, LDLc, triglycerides, pocket depth, and oral plaque level in Table 5. This table includes the key primary periodontal pathogens [*P. gingivalis, T. denticola,* and *T. forsythensis,* the Socransky Red cluster (56)], and the Orange cluster of pathogens [*P. intermedia, P. nigrescens, C. rectus, F. nucleatum,* and *P. micra*] associated with less-severe disease as well as *A. actinomycetemcomitans.* Other organisms associated with health also are included. As in Table 1, the total IgG level significantly decreases with increasing oxidative stress, even adjusting for relevant covariates. The highest quartile of oxidative stress demonstrates a significant suppression of total IgG as compared with all three lower quartiles. It can be seen across this table that for the major pathogenic organisms of the Red Cluster, Orange Cluster, and *A. actinomycetemcomitans,* a consistent trend was found for the lowest IgG titer being seen in the highest quartile of oxidative stress. The pattern is also present for the remaining biofilm inhabitants including those associated with health. The results (Table 5) demonstrate that increased serum 8-isoprostane concentrations are associated with decreased serum levels of all 17 antibodies. The serum levels of the respective antibodies differed somewhat in apparent susceptibility to increasing serum 8-isoprostane concentrations, with the serum levels of IgG antibodies for *P. gingivalis, P. intermedia, P. nigrescens, P. micra, S. intermedius, S. oralis, S. sanguis,* and *S. noxia,*

TABLE 5. ADJUSTED SERUM IgG ANTIBODY LEVELS FOR TOTAL AND INDIVIDUAL ORAL BIOFILM BACTERIA
ACCORDING TO QUARTILES OF SERUM 8-ISOPROSTANE

	Mean (SE) serum IgG antibody levels (ng/ml) by quartiles of serum 8-isoprostane				p Values for indicated comparison of 8-isoprostane quartiles		
IgG antibody specificity (n)	1	2	3	4	Q1 vs. Q4	Q2 vs. Q4	Q3 vs. Q4
Total biofilm IgG (4,080)	1.584 (45)	1,313 (44)	1,068 (44)	871 (46)	<0.0001	<0.0001	0.002
P. gingivalis (4,230)	117.2 (7.8)	69.0 (7.9)	72.8 (7.8)	56.1 (7.9)	<0.0001	0.24	0.13
T. forsythensis (4,107)	49.0 (2.3)	48.2 (2.3)	39.9 (2.3)	36.3 (2.4)	0.0002	0.0005	0.28
T. denticola (4,229)	38.2 (1.3)	37.6 (1.3)	30.2 (1.3)	23.3 (1.4)	<0.0001	<0.0001	0.0003
Total Red biofilm IgG (4,106)	205.9 (9.7)	155.2 (9.7)	144.6 (9.6)	116.7 (10.0)	<0.0001	0.006	0.04
P. intermedia (4,230)	122.2 (4.7)	90.2 (4.7)	71.9 (4.6)	59.6 (4.7)	<0.0001	<0.0001	0.06
P. nigrescens (4,230)	214.1 (7.3)	162.1 (7.3)	120.2 (7.3)	88.6 (7.3)	<0.0001	<0.0001	0.002
C. rectus (4,230)	38.8 (2.0)	36.9 (2.0)	29.9 (2.0)	27.5 (2.0)	<0.0001	0.001	0.41
F. nucleatum (4,213)	19.8 (1.1)	22.6 (1.1)	13.8 (1.1)	13.3 (1.1)	<0.0001	<0.0001	0.76
P. micra (4,230)	112.9 (5.3)	120.7 (5.3)	93.5 (5.3)	85.1 (5.3)	0.0003	<0.0001	0.27
Total Orange biofilm IgG (4,213)	512.1 (15.5)	432.5 (15.5)	329.2 (15.3)	274.1 (15.5)	<0.0001	<0.0001	0.01
A. actinomycetemcomitans (4,230)	163.7 (6.6)	174.9 (6.7)	123.5 (6.7)	111.4 (6.7)	<0.0001	<0.0001	0.20
E. corrodens (4,220)	19.0 (1.4)	25.9 (1.4)	16.3 (1.4)	15.7 (1.4)	0.09	<0.0001	0.75
C. ochracea (4,230)	40.2 (1.7)	39.7 (1.7)	29.9 (1.7)	27.4 (1.7)	<0.0001	<0.0001	0.30
V. parvula (4,230)	16.2 (1.0)	14.3 (1.0)	11.0 (1.0)	9.7 (1.0)	<0.0001	0.001	0.34
S. intermedius (4,230)	133.8 (8.3)	103.3 (8.3)	106.2 (8.2)	77.6 (8.3)	<0.0001	0.03	0.01
S. oralis (4,230)	107.8 (5.9)	96.7 (5.9)	54.5 (5.9)	35.6 (6.0)	<0.0001	<0.0001	0.02
S. sanguis (4,230)	94.6 (5.8)	77.7 (5.8)	84.8 (5.7)	58.1 (5.8)	<0.0001	0.02	0.001
S. noxia (4,230)	168.8 (10.3)	87.2 (10.3)	65.7 (10.2)	46.3 (10.4)	<0.0001	0.006	0.18
A. viscosus (4,221)	100.9 (6.8)	98.0 (6.8)	88.6 (6.8)	76.4 (6.8)	0.01	0.03	0.21
Other biofilm IgG (4,211)	748.8 (25.0)	619.5 (25.0)	491.9 (24.8)	381.8 (25.1)	<0.0001	<0.0001	0.002

Adjusted for race/center, age, gender, BMI, smoking, education, income, diabetes, hypertension, HDL, LDL, triglycerides, pocket depth, and oral plaque level.

showing the largest magnitudes of reduction between the first and last quartiles of serum 8-isoprostane. Thus, the suppression of IgG that is associated with high oxidative stress does not appear to be limited to any specific pathogenic or beneficial organisms present within the biofilm. Rather, the suppression of the IgG response was apparent across all the bacterial species evaluated, including bacteria within human plaque associated with periodontal health and disease. A similar analysis stratifying on periodontal status showed similar results (data not shown). These data clearly demonstrate that higher oxidative stress is strongly associated with a decrease in the IgG antibody response to the microbes within the subgingival biofilm and that the magnitude of the microbial burden and the presence of periodontal disease are, by comparison, minor modifiers of serum IgG antibody responses to the commensal oral biofilm.

Discussion

The findings indicate that higher serum 8-isoprostane concentrations are related to significantly decreased levels of serum IgG antibodies against the commensal oral flora. Although these titers reflect whole-bacterial titers, a high degree of specificity occurs in organism-specific IgG without significant cross-reactivity across various microbes under the stringency conditions of the immunobinding (57). Thus, the effect of oxidative stress on IgG responses appears to reflect a suppression of IgG across a wide range of potential antigenic

stimuli. Characterization of the immunodominant antigens of each bacterium tested, as well as measurements of IgG subclass responses, was beyond the scope of this molecular epidemiologic survey of 4,717 subjects. Nonetheless, the literature suggests that the immunodominant antigens for this wide range of bacteria include lipopolysaccharides, s-layer proteins, flagella, OMPs, and extracellular capsules (see, for example, 50). Thus, the suppression in IgG antibodies seen in the presence of high oxidative stress does not appear to be limited to specific biochemical subsets of antigens.

Variables related to serum 8-isoprostane

8-Isoprostane is a stable product of the oxidative metabolism of arachidonic acid (56), and 8-isoprostane concentrations in serum and urine have been used as markers of systemic oxidative stress (56) and are related to the accumulation of oxidized-LDL (39). The serum concentration of 8-isoprostane has been reported to be related to body mass index and race (8). In our study, serum 8-isoprostane concentrations were related to age, education, smoking, triglyceride levels, and race/center, but not to BMI. Because serum 8-isoprostane concentrations reflect whole-body lipid oxidation, including enzymatic and nonspecific free-radical lipid oxidative pathways, it appears likely that these variables and physiologic or pathologic factors (or both) that influence oxidative lipid metabolism contributed to the serum 8-isoprostane concentrations in our study sample.

Our findings further indicate that an increased prevalence of deep probing depths (periodontal disease) is associated with increased (1.6-fold) serum concentrations of 8-isoprostane (Table 1). The reasons for this association are not known. However, the epithelium lining of the periodontal pocket is adjacent to the subgingival biofilm and is structurally unique without tight junctions or keratin. Even in health, the epithelial attachment to the teeth is a site that has a natural IL-8 chemotactic gradient, which brings neutrophils into the periodontal pocket such that they flush around the teeth and appear in the saliva. Activated neutrophils within the crevice and saliva may serve to increase salivary 8-isoprostane, as recently reported by Wolfram (63). Thus, the periodontal tissues may represent a source of systemic oxidative stress because of the presence of high numbers of activated neutrophils. It is known that the presence of periodontitis is associated with lower serum concentrations of vitamin C (12), even after adjusting for smoking, suggesting that periodontal disease may pose an oxidative stress that consumes ascorbate. Also, the mucosal tissue wounding associated with deep periodontal pockets has been associated with an increased occurrence of bacteremia (38). In this regard, 8-isoprostane concentrations have been demonstrated to be increased in *Helicobacter pylori (H. pylori)*-positive subjects (16), noncirrhotic chronic hepatitis C patients (30) and cystic fibrosis patients (15), as well as animal models of endotoxemia (3). Because the presence of periodontitis can result in recurring systemic exposure to oral biofilm microorganisms (38), it is conceivable that the resulting low-level bacteremia, endotoxemia, and consequent metabolic stress could contribute to an elevated serum concentration of serum 8-isoprostane.

Serum 8-isoprostane related to serum IgG antibody levels

Our findings demonstrate that serum concentrations of 8-isoprostane above the median or the top quartile, or both, are related to decreased levels of serum IgG antibodies to a panel of oral bacteria. These findings were controlled for smoking status and other known contributors to oxidative stress (*e.g.*, diabetes, age, BMI, and blood lipids) and periodontal severity (*e.g.*, smoking, age, BMI, and education). To our knowledge, these findings represent the first demonstration of an association between serum 8-isoprostane concentrations and serum IgG antibody levels to mucosal microbes. Thus, this investigation demonstrates that systemic oxidative stress consistently suppresses serum IgG antibody responses to a comprehensive panel of naturally occurring mucosal biofilm microbes in a large, representative community-dwelling population.

Extremes of oxidative stress have been related to changes in a variety of immune-response mechanisms (28); however, our findings for the ARIC panel indicate that differences in serum 8-isoprostane concentrations across quartiles of 8-isoprostane can be related to significant differences in serum antibody levels for oral bacteria, showing a statistically significant dose–response relation. The bacteria in our panel of antibodies included gram-negative and -positive bacteria, strict anaerobes, aerobes, and facultative anaerobes, as well as species associated with periodontal disease and oral health (59). Because serum 8-isoprostane concentrations in the top quartile are related to decreased levels of all members of the antibody panel, this is not a narrow phenomenon pertaining to selected bacterial species; nor is it limited to pathogenic or invasive bacteria. These findings suggest that physiologic increases in serum 8-isoprostane concentration are associated with marked attenuation of serum IgG antibody responses to many species of oral bacteria for large segments of the adult population. One limitation of this analysis, however, is that we have not assessed whether the apparent decrease in titer is due to differences in IgG avidity for oral organisms.

It could be hypothesized that infiltrating neutrophils or macrophages, or both, capable of mounting an oxidative burst might control the periodontal microflora and thereby limit consequent serum IgG antibody responses, leading to an inverse relation between serum 8-isoprostane and IgG antibody levels. For a number of reasons, it is apparent that this is not the case. An increased presence of periodontal pockets of PD larger than 5 mm would provide a greater reservoir of activated periodontal PMNs and macrophages and was associated here with increased serum 8-isoprostane levels; however, the greater number of deeper pockets also were associated with significant increases in levels of serum IgG antibodies. Increased extent PD >5 mm appeared to have a smaller impact on serum 8-isoprostane levels than did serum triglycerides, education, race/center, or age. Also, suppression was not exclusive to IgG antibodies directed to bacteria associated with periodontal disease or bacterial species (*e.g.*, strict anaerobes) highly susceptible to the effects of oxidative stress. Rather, increased serum 8-isoprostane was associated with marked reductions in serum levels of IgG antibodies to plaque bacteria associated with periodontal health (*e.g.*, *S. sanguis*), which are adept at aerobic growth, and quartiles of increasing serum 8-isoprostane were associated with suppression of IgG antibody levels in subjects with both high and low levels of plaque or PD or both. The ability to mount an oxidative burst by neutrophils/macrophages is common in the adult population (41) and is not likely to distinguish among quartiles of serum 8-isoprostane levels in a broad population or of the observed association of serum 8-isoprostane with the suppression of IgG antibody responses.

In parallel analyses not reported here, we found that within this same subject population, the serum levels of IgG antibodies to the gastric bacterium *H. pylori* also were decreased significantly across quartiles of increasing serum 8-isoprostane, after controlling for variables related to oxidative stress and periodontal severity. Thus, these findings of the suppression of systemic IgG antibody responses in association with increased 8-isoprostane levels likely are not unique to oral bacteria, nor is it likely that the suppression was the consequence of a feedback loop between a control of plaque microorganisms by an oxidative burst by neutrophils/macrophages within the periodontal tissues and consequent systemic antibody responses.

Smoking has been associated with decreased levels of serum IgG antibodies to oral bacteria (60) and decreased serum concentrations of the water-soluble antioxidant ascorbate (58). Smoking also was a significant contributor to our bivariate models of serum IgG antibody levels (Table 1). In several clinical populations, serum 8-isoprostane concentrations have been reported to be related to a history of prolonged heavy smoking (46), but a correlation with smoking history was not observed in other populations (8). Because smoking has been shown not to be related to decreased serum

concentrations of the lipid-soluble antioxidant, α-tocopherol (44), it appears that smoking may have greater effects on water-soluble markers of oxidative stress than on markers within the lipid-soluble compartment. For these reasons, we included smoking history as a covariate in our models (Tables 2–5); however, excluding smoking did not have a significant effect on model parameters. In analyses not reported here among the subset of subjects who were "never smokers," serum 8-isoprostane levels had the same significant relation to serum IgG antibody levels as those reported here for the whole population. Thus, the findings reported here suggest a possible role for lipid-mediated oxidative stress in modulating the effects of exposure to the oral biofilm microflora on serum antibody levels.

The results presented in Tables 2 through 4, although not from a longitudinal study, suggest that the ability to mount an increased IgG antibody response to either an increased plaque biofilm burden (PI) or the extent of periodontal disease (extent PD greater than 5 mm) is most marked and significant among that quartile with the lowest level of serum 8-isoprostane. Conversely, physiologic increases in oxidative stress in the three upper quartiles suppressed IgG antibody levels, irrespective of the extent of plaque burden or deep probing depths.

Psychological stress, infection-induced stress, and chemically induced oxidative stress have been associated with decreased antibody levels in both animal models of infection and in various human populations (4, 18, 61). Moreover, considerable evidence indicates that redox signaling, oxidative stress, and cellular antioxidant levels can influence the function of each of the cell types (antigen-presenting cells, T cells, and B cells) regulating immunogenicity and their roles in IgG synthesis. Thus, the mechanism of suppression of serum IgG antibody levels reported here conceivably could be due to the inhibition of the function of any one of those cells or a combination of such cells or both. At the clinical level, the effects of oxidative stress appear most readily explained by an inhibition of Th1 cell function. Antigen-presenting cells (APCs) have a pivotal role in defining the balance of T-cell responses to either Th1 or Th2 cytokine profiles. Oxidative stress has been shown to impair APC signaling to T cells (54), whereas depletion of intracellular APC glutathione (GSH) was shown to favor expression of Th2 cytokines (47, 53). Studies on T cell–receptor signaling (11) and of lymphocytes in HIV/AIDS subjects (28) suggest that Th1-cell function is more susceptible to oxidative stress than is Th2-cell function, whereas oxidative stress has been related to increased Th2-driven responses that were attenuated *in vivo* with antioxidant interventions (64). Overall, it appears that oxidative stress is associated with suppression of Th1-mediated responses and increased Th2-mediated responses. Recent findings indicate that Nrf2, a redox-sensitive transcription factor that enhances expression of antioxidant genes, has an important role in the molecular mechanism whereby oxidative stress modifies Th1 responses mediated by APCs (34). In preclinical testing, Nrf2 pathway activation in dendritic cells enhanced Th1 immunity (34), whereas disruption of Nrf2 expression promoted prooxidative dendritic cells that led to increased Th2 responses (62). Other studies suggest a role for Nrf2 in modification of mucosal tissue defenses (2) in humans. Likely the impact of systemic oxidative stress on APCs, suppression of Th1 function, and mucosal defenses depends at least in part on how well signaling and gene activation mediated by Nrf2 compensate for effects of oxidative stress.

Understanding the role of oxidative stress in relation to suppression of Th1-mediated serum IgG responses likely would include an understanding of the IgG-subclass responses. The IgG subclasses (*i.e.*, IgG1, IgG2, IgG3, and IgG4 in humans) are produced in response to different classes of antigens and are differentially expressed as a function of disease stage (35). The dominant serum IgG subclass response to oral biofilm is of the IgG2 subclass. In turn, the lower total IgG titers seen in subjects with elevated 8-isoprostane may be attributable to decreased levels of IgG2 antibody.

Synthesis of the respective IgG antibody subclasses appear to be differentially regulated by Th1 and Th2 cytokine responses (19). IgG2 antibody expression has been demonstrated to be dependent on Th1 cytokines (33), whereas IgG4 expression is induced by Th2 cytokines (52). The roles of Th1 and Th2 cytokines in IgG1 and IgG3 expression are more complex (9) and appear to depend on both the type of antigen and the mode of exposure to the immune system.

Stress and mediators of oxidative stress have been associated with regulation of isotype-specific and IgG subclass–specific antibody synthesis. In mice, dietary consumption of oxidized oil has been shown to increase production of the Th2 cytokine IL4, as compared with the Th1 cytokine γ-interferon and to increase Th2-dependent IgG subclass levels and IgE antibody responses to ovalbumin (37). In humans, intense physical training (22) and chronic psychological stress have been associated with decreased salivary IgA levels. Likewise, the increase in plasma IgG2 levels typically associated with periodontitis was not observed in smokers (24), suggesting that the oxidative stress associated with smoking attenuated the IgG2 antibody responses to oral bacteria. Investigation of the relation of serum 8-isoprostane concentrations to serum levels of specific IgG antibody subclasses will be an important next step.

Conclusions

Our findings demonstrate that serum IgG antibody responses to the oral biofilm microflora are suppressed in the presence of systemic oxidative stress. Additional studies are needed to clarify the role of IgG subtype and avidity to these findings. These findings suggest possible studies to explore the clinical impact of oxidative stress in mucosal disease progression, in the response to therapy, and in the potential use of antioxidant therapies as modifiers. To understand the mechanism for the association of 8-isoprostane concentrations with decreased IgG antibody levels, it will be necessary to define better the specific mediators of lipid-based redox signaling, specific cellular and molecular targets, and correlations of IgG subclass antibody levels (and antibody isotypes) to serum 8-isoprostane concentrations. It is expected that clinical and mechanistic insights from these investigations will enable interventions that better target populations and mechanisms likely to have impact on the systemic diseases that have been associated with systemic exposure to the mucosal biofilm microflora (5, 6).

Author Disclosure Statement

No competing financial interests exist.

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Abbreviations Used

APC = antigen-presenting cell
ARIC = atherosclerosis risk in
communities study
BMI = body mass index
PD = periodontal probing depth,
measured in millimeters
CEJ = cementoenamel junction
CAL = clinical attachment level,
measured in millimeters
GSH = glutathione
HDLC = high-density lipoprotein cholesterol
LDLC = low-density lipoprotein cholesterol
LPS = lipopolysaccharide
Nrf2 = Nuclear factor erythroid 2
p45-related factor 2
OMP = outer membrane protein
PI = Plaque Index, a measure
of dental plaque accumulation
along the gum line
D-8-iso PGF$_{2\alpha}$ = 8-isoprostane or Direct-8-iso-
Prostaglandin F$_{2\alpha}$
AP = alkaline phosphatase
P. gingivalis = *Porphyromonas gingivalis*
P. intermedia = *Prevotella intermedia*
P. nigrescens = *Prevotella nigrescens*
T. forsythensis = *Tannerella forsythensis*
T. denticola = *Treponema denticola*
F. nucleatum = *Fusobacterium nucleatum*
A. actinomycetemcomitans = *Aggregatibacter*
actinomycetemcomitans
C. rectus = *Campylobacter rectus*
E. corrodens = *Eikenella corrodens*
P. micra = *Parvimonas micros*
V. parvula = *Veillonella parvula*
C. ochracea = *Capnocytophaga ochracea*
S. noxia = *Selenomonas noxia*
A. viscosus = *Actinomyces viscosus*
S. intermedius = *Streptococcus intermedius*
S. sanguis = *Streptococcus sanguis*
S. oralis = *Streptococcus oralis*
H. pylori = *Helicobacter pylori*

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