J Clin Periodontol 2010; 37: 685–696 doi: 10.1111/j.1600-051X.2010.01592.x

Journal of Clinical Periodontology

Determinants of serum IgG responses to periodontal bacteria in a nationally representative sample of US adults

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Vlachojannis C, Dye BA, Herrera-Abreu M, Pikdöken L, Lerche-Sehm J, Pretzl B, Celenti R, Papapanou PN. Determinants of serum IgG responses to periodontal bacteria in a nationally representative sample of US adults. J Clin Periodontol 2010; 37: 685–696. doi: 10.1111/j.1600-051X.2010.01592.x

Abstract

Aim: To assess the distribution of elevated antibody titres to multiple periodontal bacteria, including established/putative pathogens and health-related species, by selected demographic, behavioural, and oral- and general health-related characteristics.

Methods: Data from 8153 ⩾40-year-old participants from the third National Health and Nutrition Examination Survey were used, including 1588 edentulous individuals. We used checkerboard immunoblotting to assess serum IgG levels to 19 periodontal species. Thresholds for elevated antibody responses were defined for each species using the 90th percentile titre in periodontal healthy participants, using two alternative definitions of periodontitis.

Results: Edentulous individuals showed lower antibody responses than dentate participants, notably for titres to ''red complex'' species and *Actinobacillus actinomycetemcomitans*. Elevated titres to *Porphyromonas gingivalis* were twice as prevalent in participants with periodontitis than in periodontal healthy individuals. Non-Hispanic blacks and Mexican-Americans were more likely to display elevated titres for *P. gingivalis* compared with non-Hispanic whites (22.9% *versus* 19.4% *versus* 9.5%). Current smokers were significantly less likely to exhibit high titres to multiple bacteria than never smokers.

Conclusion: Demographic, behavioural, and oral- and general health-related characteristics were strong determinants of systemic antibody responses to periodontal bacteria in a nationally representative sample of US adults.

Key words: edentulism; IgG antibodies; NHANES; periodontitis; serum

Accepted for publication 1 May 2010

Conflict of interest and source of funding statement

The authors declare no conflict of interests. The study was supported by an American Heart Association grant to Dr. Papapanou (Grant-In-Aid #256205T) and a CTSA Award from the National Institutes of Health (#RR025158).

Disclaimer statement: The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

It is well established that periodontitis is associated with the development of a systemic antibody response (Ebersole et al. 1987, Ebersole, 1990, Papapanou et al. 2000), and elevated serum antibodies to multiple periodontal species have been reported in individuals with chronic periodontitis (Haffajee et al. 1995), early-onset periodontitis (Albandar et al. 2001), and refractory periodontitis (Hernichel-Gorbach et al. 1994, Colombo et al. 1998). In an earlier longitudinal study (Papapanou et al. 2004), we demonstrated that patients with periodontitis displayed serum IgG antibody patterns that were distinct from individuals with no or minimal periodontal tissue breakdown, and that these titres remained relatively stable over time, even after the provision of periodontal therapy. We subsequently hypothesized that serum antibody responses to periodontal bacteria may serve as surrogate markers of periodontal status,

similar to the use of serological markers in the diagnosis of history of other bacterial (Steingart et al. 2007, Tsang et al. 2007), fungal (Quindos et al. 2004), and viral (Pawlotsky 1999) infections.

To investigate this possibility, we analysed serum samples from a large-scale, nationally representative epidemiologic study (the Third National Health and Nutrition Examination Survey; NHANES III) with respect to IgG antibodies to a battery of periodontal species, and reported that the antibody titres, in combination with select demographic and behavioural characteristics resulted in moderately accurate classifications of periodontal status (Dye et al. 2009). However, several host-related characteristics and a number of environmental factors have been suggested to influence the ability of the host to mount a systemic antibody response during the course of an infection. Indeed, both the periodontal and the medical literature indicate that race (Gunsolley et al. 1988, 1991), age (Shackelford et al. 1985), and smoking (Gulsvik & Fagerhoi 1979, Quinn et al. 1996, Tangada et al. 1997, Mooney et al. 2001, Moszczynski et al. 2001) are important determinants of the systemic antibody response.

To the best of our knowledge, the effects of demographic, behavioural, and oral- and general health-related characteristics on the levels of serum IgG antibodies to multiple periodontal bacteria have not been systematically explored in a nationally representative sample of adults. This present report complements our recently published NHANES III-based serological study (Dye et al. 2009) and analyses the distribution of elevated antibody titres to periodontal bacteria by selected characteristics associated with periodontal disease, in an expanded cohort that also includes a large sample of edentulous individuals.

Methods

Data source

Data from NHANES III, 1988–1994 were used. Conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention, NHANES III utilized a complex, highly stratified, multistage probability design capable of producing a nationally representative sample. Details of the sample design and methods used in obtaining informed consent from study participants have been described elsewhere (Ezzati et al. 1992, US Department of Health and Human Services, 1994). NHANES III over sampled individuals who were either under the age of six or over the age of 60, Mexican Americans, and non-Hispanic blacks. This was performed to enhance the reliability of prevalence estimates for these groups in the non-institutionalized civilian population of the United States.

For this report, smoking history, diabetic, and socio-demographic data were obtained using a household interview questionnaire. Dental and periodontal status data were obtained by a standardized oral health examination and periodontal antibody information was obtained from a NHANES III periodontal pathogens surplus sera assessment. The dental examinations were conducted by trained dentists who were periodically calibrated by the survey's expert dental examiner and examiner reliability statistics for the periodontal exam were considered to be good (Winn et al. 1999, Kingman & Albandar 2002). Additional information regarding oral health assessment procedures and quality control has been published elsewhere (Drury et al. 1996). All data used for this report are available at http://www.cdc.gov/nchs/about/major/nhanes/datalink.htm

Study population

Overall, there were 14,464 persons aged 40 years or older who were eligible to participate in NHANES III. Of the eligible sample, 11,448 were interviewed, 9319 were examined, and 8153 had surplus sera analysed for IgG antibodies to 19 oral bacterial species. For this report, we used data from 7409 participants aged 40 years and older for whom a complete set of serum IgG antibody and relevant dental examination data were available. Among these, 1588 were edentulous and 5821 persons were dentate and had a periodontal examination. These 7409 persons represent 51.2% of the sample in this age range who were selected for NHANES III, 64.5% who were interviewed, 79.5% who were examined, and 90.9% who had surplus sera evaluated for IgG antibodies to periodontal bacteria.

Most sampled persons participating in phlebotomy in the MEC had additional blood drawn to be stored for future laboratory tests. Among this group, 8153 persons had surplus sera analysed for IgG antibodies to 19 oral bacterial species. These analyses were performed by the Oral and Diagnostic Sciences Laboratory, Columbia University College of Dental Medicine, using the ''checkerboard'' immunoassay technique (Sakellari et al. 1997, Papapanou et al. 2000) and have been described in detail in an earlier publication (Dye et al. 2009). Additional background information regarding the NHANES III periodontal antibody data has been published elsewhere: ftp://ftp.cdc.gov/pub/Health_Statistics/NCHS/Datasets/NHANES/NHANESIII/30a/spsdeppx.pdf.

Variable definition

For these analyses, we included a number of demographic and health status indicators that are known to be associated with periodontal status. Race/ethnicity was categorized as Mexican-American, non-Hispanic black, non-Hispanic white, and other. Although specific estimates for ''others'' are not presented in all tables in this report, ''others'' were included in the analyses of the total study population. Education attainment was categorized as not completing high school, completed high school, or having at least some college experience. Age and sex were also analysed. Age was categorized into three age groups: 40–54-year-olds, 55–69-year-olds, and persons aged 70 years or older.

Cigarette smoking was categorized as either current smoker, former smoker, or never smoked. Individuals who reported that they had smoked at least 100 cigarettes (approximately five packs) in their lifetime but no longer smoked cigarettes were classified as former smokers. Individuals were classified as having diabetes mellitus, if they reported being diagnosed with diabetes by a physician. Women who may have had only gestational diabetes were classified as being non-diabetic. Dental status was categorized into four groups: edentulous, having 1–9 teeth, 10–19 teeth, and 20 or more teeth.

We used two alternative definitions for pathological periodontal status for these analyses. Individuals were classified as having moderate or severe periodontitis (Definition #1) using the guidelines for assessing periodontal status in population studies proposed by

the CDC and the American Academy of Periodontology (AAP) (Page & Eke 2007). In brief, a person was determined to have severe periodontitis if there were two or more teeth with interproximal attachment loss (AL) $\geqslant$6 mm and one or more teeth with interproximal pocket depth (PD) $\geqslant$5 mm. Moderate periodontitis existed if there were two or more teeth with interproximal AL$\geqslant$4 mm or two or more teeth with interproximal PD$\geqslant$5 mm. According to an alternative definition (Definition #2), individuals were classified as having extensive periodontitis if interproximal AL$\geqslant$5 mm affected at least 30% of the teeth present (Tonetti & Claffey 2005). Persons who had neither moderate/severe periodontitis according to Definition #1 nor extensive periodontitis according to Definition #2 were determined as periodontal healthy according to both definitions (Periodontal healthy). For each of the antibody titres assessed, we determined ''elevated'' antibody status by using the 90th percentile titre level as a cut-off point in individuals without periodontal disease (Periodontal healthy). These threshold levels were determined separately for each of the two NHANES III data collection phases, and the rationale for doing so was described earlier (Dye et al. 2009).

Table 1. Dental status by selected demographic and health characteristics for adults aged 40 years and older†: United States, 1988–1994

Characteristics	*n*	Dental status	
		edentulous, % (SE)	dentate, % (SE)
Age			
70 years or older	2410	34.2 (2.0)*	65.8 (2.0)
55–69 years	2700	21.4 (1.4)*	78.6 (1.4)
40–54 years[R]	3043	6.9 (0.9)	93.9 (0.9)
Gender			
Male	3796	16.1 (1.0)	83.9 (1.0)
Female[R]	4357	17.9 (1.0)	82.1 (1.0)
Race/ethnicity			
Mexican-American	1779	6.8 (0.6)*	93.3 (0.6)
Non-Hispanic black	1921	16.3 (1.0)	83.7 (1.0)
Non-Hispanic white[R]	4126	18.1 (1.1)	81.9 (1.1)
Other	327	11.8 (2.1)	88.2 (2.1)
Education attainment			
Not completed high school	3746	32.1 (1.7)*	67.9 (1.7)
Completed high school	2265	16.2 (1.2)*	83.8 (1.2)
Some college[R]	2084	6.4 (0.9)	93.6 (0.9)
Information not reported	58	–	–
Cigarette smoking			
Current smoker	1803	21.7 (1.6)*	78.3 (1.6)
Former smoker	2645	19.0 (1.3)*	81.0 (1.3)
Never smoked[R]	3705	13.2 (1.1)	86.8 (1.1)
Diagnosed with diabetes			
Yes	967	29.7 (1.8)*	70.3 (1.8)
No[R]	7186	16.0 (1.0)	84.0 (1.0)
Total	8153	17.1 (0.9)	82.9 (0.9)

Source: third National Health and Nutrition Examination Survey (NHANES III).
†For individuals with available serum antibody titres to periodontal bacteria.
*p-value <0.05 compared with the reference group.
%, weighted percent; SE, weighted standard error; R, reference group; DSU, data statistically unreliable.

Data analysis

All statistical analyses were performed using SUDAAN (version 9.0, RTI, Research Triangle Park, NC, USA), a statistical software designed to accommodate complex sample surveys. Sample weights were used to account for the unequal probability of selection and non-response of the study participants to produce prevalence estimates and related standard errors. The statistical significance of differences between estimates was evaluated using two-sided *t*-tests ($p<0.05$). These tests were conducted without adjustment for other covariates, or multiple comparisons.

Multivariable logistic regression (MLR) modelling was used to assess the relationship between elevated titre status and key covariates for both dentate and edentulous study participants. Although MLR models were produced for each titre and included all of the covariates, estimates from only those covariates that were statistically significant ($p<0.05$ using the Wald *F*-statistic) are presented in Tables 5 and 6.

Results

Table 1 shows the distribution of the study sample according to dental status and a number of demographic and health characteristics. The prevalence of edentulous individuals was approximately 17% (weighted%). Edentulism increased with age, was significantly lower in Mexican-Americans than in other race/ethnicity groups, and was more pronounced in individuals with no college education, in current and former smokers, and in individuals diagnosed with diabetes.

Supporting information Table S1, available in the online publication, describes the prevalence of periodontal disease in the healthy participants, according to two alternative definitions, as well as the prevalence of healthy periodontal conditions, by selected demographic and health characteristics. Table 2 describes the prevalence of elevated antibody titres by periodontal status. Only the prevalence of elevated titres to *Porphyromonas gingivalis* was statistically significantly different between periodontal healthy and diseased participants across all definitions.

The prevalence of elevated antibody titres by dental status is shown in Table 3. Edentulous individuals exhibited a significantly lower prevalence of elevated antibody titres to 11 periodontal bacteria (*Actinobacillus actinomycetemcomitans*, *P. gingivalis*, *Tannerrella forsythia*, *T. denticola*, *Campylobacter rectus*, *Eubacterium nodatum*, *Prevotella intermedia*, *Prevotella nigrescens*, *Fusobacterium nucleatum*, *Selenomonas noxia*, and *Capnocytophaga ochracea*) compared with dentate participants. Additionally, the prevalence of elevated antibody titres was significantly lower for persons with one to nine remaining teeth for antibodies to six periodontal bacteria (*C. rectus*, *E. nodatum*, *P. nigrescens*, *S. noxia*, *C. ochracea*, and *S. mutans*) compared with those with 20 or more teeth. Individuals with only 10–19 natural teeth were more likely to have elevated antibodies to *A. actinomycetemcomitans* and *Staphylococcus intermedius* compared with those with 20 or more teeth.

Table 2. Prevalence of elevated antibody titres, according to two alternative disease definitions, among persons aged 40 years and older*: United States, 1988–1994

Titres	Definition 1		Definition 2		Periodontal healthy according to both definitions	
	yes, % (SE)	no^R, % (SE)	yes, % (SE)	no^R, % (SE)	yes^R, % (SE)	no, % (SE)
A. actinomycetemcomitans Mix[1]	11.2 (1.6)	10.0 (1.0)	8.5 (1.9)	10.4 (1.0)	10.0 (1.6)	11.3 (1.6)
A. actinomycetemcomitans 29523	12.3 (1.4)	10.2 (1.0)	11.2 (2.1)	10.5 (1.0)	10.1 (1.0)	12.5 (1.4)
A. actinomycetemcomitans Y4	12.2 (1.4)	10.2 (0.7)	13.2 (3.5)	10.4 (0.7)	10.0 (0.8)	13.0 (1.9)
P. gingivalis Mix[2]	19.4 (1.4)*	10.1 (0.6)	24.3 (2.3)*	10.7 (0.6)	10.0 (0.6)	19.0 (1.3)*
T. forsythia	10.6 (1.3)	10.3 (0.9)	9.0 (1.5)	10.4 (0.8)	10.7 (1.2)	10.2 (0.9)
T. denticola	9.5 (1.5)	10.0 (1.4)	9.6 (1.8)	10.0 (1.3)	10.1 (1.4)	9.4 (1.4)
C. rectus	13.2 (1.3)*	9.9 (1.0)	11.6 (2.0)	10.4 (1.0)	10.0 (1.1)	12.5 (1.3)
E. corrodens	10.5 (1.3)	10.2 (1.3)	10.9 (2.0)	10.2 (1.3)	10.1 (1.3)	11.0 (1.3)
E. nodatum	6.7 (1.5)	10.0 (0.9)	4.8 (1.3)*	9.8 (0.8)	10.1 (0.9)	6.8 (1.4)
P. intermedia	12.9 (1.2)	10.1 (1.2)	12.9 (2.2)	10.4 (1.0)	10.0 (1.2)	13.1 (1.2)
P. nigrescens	9.6 (0.9)	10.2 (0.9)	11.0 (2.1)	10.0 (0.8)	10.2 (1.0)	10.0 (0.9)
P. melaninogenica	9.3 (1.1)	10.1 (1.0)	11.3 (2.4)	9.9 (1.0)	10.0 (1.1)	9.9 (1.2)
F. nucleatum	10.0 (1.1)	10.2 (1.1)	11.5 (1.8)	10.1 (0.9)	10.9 (1.0)	10.0 (1.0)
M. micros	12.5 (1.8)	10.3 (0.8)	12.5 (2.5)	10.5 (0.8)	10.1 (0.8)	13.3 (1.8)
S. noxia	10.9 (1.2)	10.0 (1.5)	9.8 (1.7)	10.2 (1.5)	10.0 (1.6)	10.7 (1.2)
C. ochracea	10.5 (1.8)	10.1 (1.2)	9.0 (2.1)	10.3 (1.2)	10.8 (1.8)	10.0 (1.3)
S. intermedius	14.1 (2.0)	10.1 (1.2)	11.7 (2.2)	10.7 (1.1)	10.0 (1.2)	14.1 (1.9)
S. oralis	11.5 (1.2)	10.1 (0.9)	11.6 (2.2)	10.3 (0.9)	10.0 (0.9)	11.8 (1.3)
S. mutans	11.5 (1.4)	10.0 (1.0)	9.7(2.3)	10.3 (0.9)	10.1 (1.0)	11.2 (1.3)
V. parvula	11.9 (1.4)	10.0 (1.0)	11.3 (1.7)	10.2 (1.0)	10.0 (1.0)	11.6 (1.3)
A. naeslundii	8.1 (1.5)	10.1 (1.0)	6.9 (1.5)*	9.9 (1.0)	10.1 (1.0)	8.2 (1.4)

Source: third National Health and Nutrition Examination Survey (NHANES III).

*For dentate individuals with available serum antibody titres to periodontal bacteria who completed a periodontal examination ($n = 5821$).

Definition 1: Moderate or severe periodontitis; CDC/AAP definition.

Definition 2: Extensive periodontitis; proximal AL of $\geqslant 5$ mm in 30% of teeth present.

Periodontally healthy according to both definitions: i.e., having neither moderate/severe periodontitis (Definition 1) nor extensive periodontitis (Definition 2).

Mix[1]: Titre to a mixed suspension of *A. actinomycetemcomitans* ATCC strains #43718, #29523, and #33384 (Y4).

Mix[2]: Titre to a mixed suspension of *P. gingivalis* ATCC strains #33277 and #53978.

*p-value <0.05 compared with the reference group.

%, weighted percent; SE, weighted standard error; R, reference group.

The prevalence of elevated periodontal antibody titres by key variables associated with periodontal status is presented in Table 4. Compared with individuals aged 40–54 years, individuals aged 70 years and older were more likely to have elevated antibodies to *P. gingivalis* (16.5% *versus* 10.2%) and *Veillonella parvula* (13% *versus* 9.5%), but were less likely to have elevated antibodies to *E. nodatum* (6.3% *versus* 11.4%) and *Actinomyces naeslundii* (7.3% *versus* 10.8%). Persons aged 55–69 years also were less likely to have elevated antibodies to *E. nodatum* (7.5% *versus* 11.4%) but more likely to have elevated antibodies to *A. actinomycetemcomitans Y4* (12.5% *versus* 9.5%) compared with younger adults.

Mexican-Americans were nearly twice as likely to have elevated antibodies to *P. gingivalis* compared with non-Hispanic whites (19.4% *versus* 9.5%) and also more likely to have elevated antibodies to the combination of the *A. actinomycetemcomitans* strains (10.1% *versus* 8.7%) and to *A. actinomycetemcomitans Y4* (11.9% *versus* 8.8%), to *C. rectus* (14.5% *versus* 9.4%) and to *P. intermedia* (12.9% *versus* 8.7%), when compared with non-Hispanic whites. Non-Hispanic blacks were more than twice as likely to have elevated antibodies against *P. gingivalis* compared with non-Hispanic whites (22.9% *versus* 9.5%), and to additional periodontal bacteria [*A. actinomycetemcomitans* mix (13.7% *versus* 8.7%), *A. actinomycetemcomitans Y4* (14.9% *versus* 8.8%), *C. rectus* (15.8 *versus* 9.4%), *P. intermedia* (20.2% *versus* 8.7%), *P. nigrescens* (15.4% *versus* 9.3%), *Eikenella corrodens* (14% *versus* 10%), *Streptococcus oralis* (14.1% *versus* 10.1%), and *S. mutans* (14.5% *versus* 9.9%)] but were less likely to have elevated antibodies to *Micromonas micros* (7.2% *versus* 11%).

Men were more likely to exhibit elevated antibody titres to six periodontal bacteria than females (*C. rectus*, 11.9% *versus* 9.3%; *P. intermedia*, 11.6% *versus* 9.7%; *P. nigrescens*, 11.5% *versus* 8.8%; *Prevotella melaninogenica*, 11.9% *versus* 8.3%; *F. nucleatum*, 11.9% *versus* 8.7%; and *S. noxia*, 11.5% *versus* 8.9%). Persons with some college education were significantly less likely to have elevated antibodies to *P. gingivalis* (9.5% *versus* 16.8%), *A. actinomycetemcomitans* (10.1% *versus* 12.4%), and *P. intermedia* (9.9% *versus* 12.5%) compared with those who had not finished high school. In contrast, they were more likely to have elevated antibody titres to *E. nodatum* (12.1% *versus* 6.1%), *M. micros* (11.6 *versus* 8.6%), and *A. naeslundii* (11.9% *versus* 7.2%). When comparing persons with and without diagnosed diabetes, only the prevalence of high titres to *P. gingivalis* differed significantly (16.0% *versus* 11.4%). Finally, current smokers

Table 3. Prevalence of elevated antibody titres by dental status among persons aged 40 years and older[†]: United States, 1988–1994

Titres	Total %, (SE)	Edentulous, % (SE)	Dentate[R], % (SE)	Dental status		
				1–9 teeth, % (SE)	10–19 teeth, % (SE)	20 or more teeth[R], % (SE)
A. actinomycetemcomitans Mix[1]	9.3 (0.8)	5.3 (0.8)*	10.1 (0.9)	7.9 (1.5)	11.4 (1.3)	10.3 (1.1)
A. actinomycetemcomitans 29523	9.6 (0.7)	5.8 (0.6)*	10.4 (0.9)	8.8 (1.3)	12.5 (1.4)	10.3 (1.1)
A. actinomycetemcomitans Y4	9.9 (0.6)	7.2 (0.9)*	10.4 (0.6)	11.5 (2.3)	13.0 (1.1)*	9.8 (0.9)
P. gingivalis Mix[2]	10.1 (0.5)	3.0 (0.5)*	11.5 (0.6)	8.9 (1.2)	14.2 (1.2)	11.5 (0.7)
T. forsythia	9.4 (0.7)	4.7 (0.8)*	10.3 (0.8)	7.8 (1.5)	9.5 (1.2)	10.9 (1.0)
T. denticola	9.2 (1.3)	6.1 (1.2)*	9.9 (1.4)	7.6 (1.3)	8.9 (1.4)	10.6 (1.5)
C. rectus	9.3 (0.8)	3.9 (0.8)*	10.5 (0.9)	5.0 (1.0)*	10.9 (1.3)	11.2 (1.1)
E. corrodens	10.5 (1.2)	10.2 (1.7)	10.5 (1.2)	10.6 (2.0)	10.8 (1.5)	10.1 (1.2)
E. nodatum	8.6 (0.6)	5.8 (1.0)*	9.2 (0.7)	7.3 (1.3)*	5.2 (0.9)*	10.9 (0.9)
P. intermedia	9.4 (0.9)	4.9 (0.7)*	10.4 (1.0)	9.4 (1.5)	12.3 (1.1)	10.4 (1.3)
P. nigrescens	9.3 (0.6)	5.5 (0.6)*	10.1 (0.7)	8.7 (0.9)*	9.7 (1.0)	10.4 (0.9)
P. melaninogenica	9.6 (0.9)	8.5 (1.3)	9.8 (1.0)	10.2 (1.7)	9.3 (1.1)	10.1 (1.1)
F. nucleatum	9.5 (0.8)	6.6 (0.9)*	10.0 (0.8)	8.3 (2.0)	11.1 (1.3)	10.3 (1.0)
M. micros	10.6 (0.7)	11.4 (1.1)	10.5 (0.7)	11.4 (2.0)	11.4 (1.3)	10.4 (0.8)
S. noxia	9.7 (1.3)	6.9 (1.3)*	10.3 (1.4)	6.7 (1.3)*	11.6 (1.5)	10.3 (1.6)
C. ochracea	9.3 (1.0)	5.2 (1.1)*	10.2 (1.1)	6.9 (1.5)*	9.9 (1.4)	10.7 (1.3)
S. intermedius	10.2 (0.9)	8.1 (1.1)	10.6 (1.0)	9.4 (1.5)	13.8 (1.5)*	10.3 (1.3)
S. oralis	10.1 (0.8)	9.0 (1.3)	10.3 (0.9)	8.9 (2.1)	11.1 (1.2)	10.4 (0.9)
S. mutans	9.9 (0.9)	8.5 (1.1)	10.2 (0.9)	7.0 (1.4)*	10.7 (1.3)	10.7 (1.0)
V. parvula	10.1 (0.8)	8.5 (1.1)	10.4 (0.9)	8.2 (1.3)	11.5 (1.3)	10.3 (1.0)
A. naeslundii	9.3 (0.9)	7.6 (1.5)	9.6 (0.9)	8.4 (1.3)	6.2 (1.4)*	10.8 (1.2)

Source: third National Health and Nutrition Examination Survey (NHANES III).

[†]For all individuals with available serum antibody titres to periodontal bacteria ($n = 8153$).

Mix[1]: Titre to a mixed suspension of *A. actinomycetemcomitans* ATCC strains #43718, #29523, and #33384 (Y4).

Mix[2]: Titre to a mixed suspension of *P. gingivalis* ATCC strains #33277 and #53978.

*p-value <0.05 compared with the reference group.

%, weighted percent; SE, weighted standard error; R, reference group.

were significantly less likely to exhibit high titres to nine periodontal bacteria than never smokers (*P. gingivalis*, 8.6% *versus* 13.1%; *T. forsythia*, 6.6% *versus* 11.5%; *Treponema denticola*, 7.8% *versus* 10.6%; *C. rectus*, 6.4% *versus* 12.5%; *E. nodatum*, 6.7% *versus* 10.7%; *P. nigrescens*, 6.8% *versus* 11.0%; *P. melaninogenica*, 6.3% *versus* 9.9%; *V. parvula*, 5.9% *versus* 11.5%; and *A. naeslundii*, 6.9% *versus* 11.6% but significantly more likely to show high titres to *M. micros* (15.0% *versus* 8.0%).

The association between elevated titre status and key covariates for dentate adults is shown in Table 5. In models accounting for socio-demographic, health, and behaviour covariates, non-Hispanic blacks were more likely to have elevated systemic antibody responses to multiple periodontal bacteria (13 titres in total) compared with non-Hispanic whites. However, Mexican-Americans were more likely to have elevated titres to three periodontal bacteria (*P. gingivalis*, *C. rectus*, and *P. intermedia*) and were less likely to have elevated antibodies to *V. parvula* compared with non-Hispanic whites. Men were approximately 50% more likely to have elevated titres to four periodontal bacteria (*C. rectus*, *P. nigrescens*, *P. melaninogenica*, and *F. nucleatum*) and were approximately 40% less likely to have elevated titres to *A. naeslundii* (OR = 0.6; 95% CI: 0.4, 0.9) compared with women. Current smokers were less likely to have elevated antibodies to seven periodontal bacteria, including *P. gingivalis* (OR = 0.6; 95% CI: 0.5, 0.9), but were at least twice as likely to have elevated antibodies to *M. micros* (OR = 2.2; 95% CI: 1.5, 3.3) compared with non-smokers. Persons with elevated titres to *P. gingivalis* were more likely to be 70 years and older (OR = 1.9; 95% CI: 1.4, 2.6), be Mexican-American (OR = 2.1; 95% CI: 1.5, 2.9), be non-Hispanic blacks (OR = 3.0; 95% CI: 2.4, 3.7), and to not have finished high school (OR = 1.5; 95% CI: 1.1, 2.1). Moreover, they were less likely to be smokers (OR = 0.6; 95% CI: 0.5, 0.9) or have one to nine teeth (OR = 0.7; 95% CI: 0.5, 0.9).

Table 6 shows the association between elevated titre status and key covariates in edentulous adults. In similar models accounting for socio-demographic, health, and behavioural variables, non-Hispanic blacks were more likely to have elevated titre status to 11 periodontal bacteria and Mexican-Americans were more likely to have four elevated titres compared with non-Hispanic whites. Among edentulous adults, the magnitude of the association between elevated *P. intermedia* antibodies was higher among Mexican-Americans (OR = 4.7; 95% CI: 2.3, 9.4) compared with non-Hispanic blacks (OR = 3.9; 95% CI: 2.2, 6.8). Edentulous men were more likely to have elevated titres to two periodontal bacteria (*P. gingivalis* and *F. nucleatum*) compared with edentulous women, and current/former smoking status was associated with elevated antibodies to four periodontal bacteria (*A. actinomycetemcomitans Y4*, *E. corrodens*, *M. micros*, and *S. oralis*). Unlike dentate adults not finishing high school, edentulous adults with low educational attainment were more than three times as likely to have elevated antibodies to *A. actinomycetemcomitans* mix (OR = 3.4; 95% CI: 1.2, 9.3).

Discussion

We determined serum IgG antibody levels to a battery of periodontal bacteria, including established, putative, and health-associated species, in a nationally representative sample of US adults comprised of 8153 individuals. In this study, we explored the distribution of elevated titres with respect to dental and periodontal status, as well as to a number of demographic, behavioural, and health-related characteristics and identified a number of significant determinants of the systemic antibody response to the plaque biofilm.

In our analyses, we established the threshold for an elevated antibody titre against each of the 19 species as the 90th percentile value in participants who were deemed to be ''periodontal healthy''. In turn, the latter group was defined using two alternative definitions of periodontitis (the CDC/AAP definition for moderate or severe periodontitis (Page & Eke 2007) and the one for extensive periodontitis (Tonetti & Claffey 2005) and included individuals who did not fulfill the periodontitis criteria for both definitions. We point to the fact that these threshold values are different from those used in our recent publication (Dye et al. 2009), where we purposefully optimized the cut-off points to maximize the sensitivity and specificity of a serology-based diagnostic test for periodontitis. However, for the purposes of the present descriptive analyses, we considered that a percentile-derived

Table 4. Prevalence of elevated antibody titres by selected socio-demographic, health, and behaviour characteristics for persons aged 40 years and older†: United States, 1988–1994

Titres	Total, % (SE)	Age group			Race/ethnicity		
		70 years/older, % (SE)	55–69 years, % (SE)	40–54 years[R], % (SE)	Mexican-Americans, % (SE)	NH-blacks, % (SE)	NH-whites[R], % (SE)
A. actinomycetemcomitans Mix[1]	10.2 (0.9)	8.3 (1.0)	11.7 (1.1)	10.0 (1.4)	10.1 (1.2)*	13.7 (1.2)*	8.7 (0.9)
A. actinomycetemcomitans 29523	10.6 (0.9)	9.8 (1.1)	13.7 (1.3)*	9.1 (1.2)	9.5 (1.5)	12.0 (1.3)	9.5 (0.9)
A. actinomycetemcomitans Y4	10.7 (0.7)	10.7 (0.9)	12.5 (1.1)*	9.5 (1.0)	11.9 (1.2)*	14.9 (1.3)*	8.8 (0.8)
P. gingivalis Mix[2]	11.7 (0.6)	16.5 (1.5)*	12.1 (0.8)	10.2 (0.8)	19.4 (1.5)*	22.9 (1.1)*	9.5 (0.7)
T. forsythia	10.3 (0.8)	11.8 (1.4)	10.5 (1.2)	9.8 (0.9)	7.4 (1.4)	12.0 (1.2)	9.9 (0.8)
T. denticola	9.9 (1.3)	8.9 (1.7)	10.0 (1.4)	10.2 (1.5)	11.4 (3.4)	12.7 (1.8)	9.7 (1.3)
C. rectus	10.5 (0.9)	13.1 (1.7)	9.7 (1.1)	10.2 (1.2)	14.5 (1.9)*	15.8 (1.3)*	9.4 (1.0)
E. corrodens	10.3 (1.2)	12.2 (1.7)	10.4 (1.3)	9.7 (1.4)	7.5 (1.3)	14.0 (1.7)*	10.0 (1.3)
E. nodatum	9.4 (0.7)	6.3 (0.8)*	7.5 (1.0)*	11.4 (1.0)	10.0 (1.0)	10.0 (0.8)	9.7 (0.9)
P. intermedia	10.6 (1.0)	11.4 (1.0)	10.2 (1.0)	10.6 (1.5)	12.9 (1.3)*	20.2 (1.3)*	8.7 (1.1)
P. nigrescens	10.1 (0.7)	12.2 (1.1)	9.8 (0.9)	9.6 (1.1)	10.2 (1.0)	15.4 (1.2)*	9.3 (0.8)
P. melaninogenica	10.0 (0.9)	10.4 (1.5)	9.2 (0.9)	10.3 (1.3)	8.3 (1.7)	10.7 (1.4)	9.9 (1.0)
F. nucleatum	10.2 (0.9)	9.3 (1.0)	10.1 (1.1)	10.5 (1.2)	9.7 (1.4)	12.3 (1.3)	9.8 (1.0)
M. micros	10.7 (0.7)	10.0 (1.0)	11.1 (0.9)	10.7 (1.1)	9.8 (1.1)	7.2 (0.9)*	11.0 (0.8)
S. noxia	10.2 (1.4)	9.2 (1.2)	10.9 (1.6)	10.0 (1.9)	11.0 (2.2)	12.0 (1.7)	9.8 (1.5)
C. ochracea	10.2 (1.2)	8.8 (1.2)	9.3 (1.2)	11.0 (1.7)	9.3 (1.6)	11.3 (1.8)	9.8 (1.3)
S. intermedius	10.8 (1.0)	10.0 (1.3)	10.1 (1.3)	11.4 (1.5)	11.2 (1.2)	13.0 (1.0)	10.3 (1.1)
S. oralis	10.4 (0.8)	10.9 (1.3)	11.4 (1.3)	9.6 (1.0)	8.7 (1.3)	14.1 (1.6)*	10.1 (0.9)
S. mutans	10.3 (0.9)	9.8 (1.0)	10.3 (1.3)	10.4 (1.1)	9.3 (1.7)	14.5 (1.6)*	9.9 (0.9)
V. parvula	10.3 (0.9)	13.0 (1.5)*	10.4 (1.1)	9.5 (1.1)	6.0 (0.8)*	9.9 (1.3)	10.1 (0.9)
A. naeslundii	9.7 (1.0)	7.3 (1.1)*	8.8 (1.1)	10.8 (1.4)	11.0 (1.0)	9.3 (1.0)	9.9 (1.2)

Titres	Gender		Education			Diagnosed with diabetes	
	male, % (SE)	female[R], % (SE)	not finished HS, % (SE)	finished HS, % (SE)	some college[R], % (SE)	yes, % (SE)	no[R], % (SE)
A. actinomycetemcomitans Mix[1]	10.3 (1.2)	10.2 (0.9)	12.4 (1.2)*	8.7 (0.9)	10.1 (1.5)	7.3 (1.9)	10.4 (0.9)
A. actinomycetemcomitans 29523	9.9 (1.1)	11.2 (0.9)	11.4 (0.9)	10.0 (1.0)	10.4 (1.6)	7.8 (1.8)	10.8 (0.9)
A. actinomycetemcomitans Y4	10.3 (0.9)	10.8 (0.8)	12.5 (1.2)	10.1 (0.9)	9.8 (1.3)	9.0 (1.8)	10.7 (0.7)
P. gingivalis Mix[2]	11.2 (0.9)	12.2 (0.8)	16.8 (1.4)*	10.8 (1.1)	9.5 (0.9)	16.0 (1.9)*	11.4 (0.6)
T. forsythia	11.0 (1.2)	9.7 (1.0)	9.2 (1.2)	10.1 (1.0)	11.0 (1.3)	10.6 (2.1)	10.3 (0.8)
T. denticola	10.3 (1.5)	9.7(1.2)	9.5 (1.3)	9.7 (1.6)	10.5 (1.5)	9.5 (1.7)	10.0 (1.3)
C. rectus	11.9 (1.1)*	9.3 (0.9)	10.6 (1.0)	8.9 (1.1)	11.4 (1.4)	13.7 (1.8)	10.3 (0.9)
E. corrodens	10.8 (1.2)	9.8 (1.3)	10.3 (1.5)	11.0 (1.6)	9.6 (1.5)	7.1 (2.0)	10.5 (1.2)
E. nodatum	9.7 (1.0)	9.2 (1.0)	6.1 (0.5)*	8.2 (1.0)*	12.1 (1.1)	7.2 (1.3)	9.6 (0.8)
P. intermedia	11.6 (1.2)*	9.7 (1.1)	12.5 (1.1)*	10.3 (1.2)	9.9 (1.7)	8.1 (1.8)	10.8 (1.0)
P. nigrescens	11.5 (0.9)*	8.8 (0.9)	10.9 (1.1)	9.8 (1.0)	9.8 (1.3)	10.3 (1.6)	10.1 (0.8)
P. melaninogenica	11.9 (1.3)*	8.3 (1.0)	8.2 (1.0)	9.3 (1.5)	11.5 (1.6)	7.2 (1.5)	10.2 (0.9)
F. nucleatum	11.9 (1.2)*	8.7 (0.9)	8.9 (0.9)	9.9 (1.2)	11.1 (1.3)	9.3 (1.6)	10.3 (0.9)
M. micros	11.4 (1.0)	10.0 (1.1)	8.6 (1.0)*	10.9 (1.4)	11.6 (1.1)	8.2 (2.2)	10.9 (0.7)
S. noxia	11.5 (1.7)*	8.9 (1.3)	10.7 (1.5)	9.1 (2.0)	10.5 (1.4)	9.7 (2.5)	10.2 (1.4)
C. ochracea	10.8 (1.4)	9.6 (1.2)	9.3 (1.4)	9.1 (1.6)	11.3 (1.4)	7.9 (1.9)	10.3 (1.2)
S. intermedius	11.6 (1.2)	10.1 (1.2)	9.4 (1.2)	11.7 (1.3)	10.8 (1.6)	9.5 (1.4)	10.9 (1.1)
S. oralis	10.7 (0.9)	10.1 (1.1)	9.4 (0.9)	10.4 (1.2)	10.9 (1.2)	12.0 (2.0)	10.3 (0.9)
S. mutans	10.5 (0.9)	10.1 (1.2)	9.4 (1.1)	10.0 (1.4)	10.8 (1.1)	8.1 (1.7)	10.4 (0.9)

Table 4. (Contd.)

Titres	Gender		Education			Diagnosed with diabetes	
	male, % (SE)	female[R], % (SE)	not finished HS, % (SE)	finished HS, % (SE)	some college[R], % (SE)	yes, % (SE)	no[R], % (SE)
V. parvula	11.1 (1.1)	9.6 (1.1)	10.0 (1.0)	10.0 (1.1)	10.6 (1.4)	9.6 (2.3)	10.4 (0.9)
A. naeslundii	9.5 (1.2)	9.9 (1.1)	7.2 (1.0)*	8.6 (1.0)*	11.9 (1.6)	9.1 (2.1)	9.8 (1.0)

Titres	Smoking status		
	current smoker, % (SE)	former smoker, % (SE)	never smoked[R], % (SE)
A. actinomycetemcomitans Mix[1]	7.2 (1.4)	11.6 (1.6)	10.6 (1.1)
A. actinomycetemcomitans 29523	7.8 (1.1)	12.5 (1.5)	10.4 (1.0)
A. actinomycetemcomitans Y4	8.7 (1.3)	11.7 (1.4)	10.6 (0.9)
P. gingivalis Mix[2]	8.6 (1.1)*	11.8 (1.3)	13.1 (0.9)
T. forsythia	6.6 (1.3)*	11.1 (1.2)	11.5 (1.2)
T. denticola	7.8 (1.5)*	10.5 (1.7)	10.6 (1.3)
C. rectus	6.4 (1.3)*	10.4 (1.3)	12.5 (1.2)
E. corrodens	8.2 (1.4)	11.2 (1.6)	10.5 (1.4)
E. nodatum	6.7 (1.2)*	9.5 (1.3)	10.7 (1.0)
P. intermedia	9.2 (1.3)	11.8 (1.9)	10.4 (1.0)
P. nigrescens	6.8 (1.2)*	10.9 (1.0)	11.0 (1.0)
P. melaninogenica	6.3 (1.6)*	12.4 (1.5)	9.9 (1.2)
F. nucleatum	7.8 (1.1)	11.3 (1.5)	10.5 (1.2)
M. micros	15.0 (1.7)*	11.5 (1.2)*	8.0 (0.8)
S. noxia	8.0 (1.6)	11.6 (2.3)	10.1 (1.3)
C. ochracea	7.9 (1.2)	10.8 (1.5)	10.7 (1.4)
S. intermedius	11.4 (1.7)	12.3 (1.7)	9.4 (1.1)
S. oralis	9.3 (1.3)	11.2 (1.2)	10.2 (0.9)
S. mutans	9.0 (1.6)	11.4 (1.4)	10.1 (1.1)
V. parvula	5.9 (0.8)*	11.4 (1.4)	11.5 (1.1)
A. naeslundii	6.9 (1.2)*	9.0 (1.3)	11.6 (1.5)

Source: third National Health and Nutrition Examination Survey (NHANES III).

†For dentate individuals with available serum antibody titres to periodontal bacteria who completed a periodontal examination ($n = 5821$).

Mix[1]: Titre to a mixed suspension of *A. actinomycetemcomitans* ATCC strains #43718, #29523, and #33384 (Y4).

Mix[2]: Titre to a mixed suspension of *P. gingivalis* ATCC strains #33277 and #53978.

*p-value <0.05 compared with the reference group.

R, reference group; %, weighted percent; SE, weighted standard error.

threshold based on antibody titres of periodontitis-free individuals was more meaningful and straightforward.

Given that our findings indicated that the prevalence of elevated antibody titres against several periodontal species was higher in non-Hispanic blacks and Mexican Americans than in non-Hispanic whites, it could be argued that the cut-off thresholds for elevated titres should be defined separately for each specific race-ethnicity subgroup. We did not elect to adopt this approach for the following main reasons: First, the generated threshold values were not intended for diagnostic purposes or clinical applications, but rather were generated to identify differences among subgroups and to explore potential reasons for these differences in the setting of a large epidemiologic study. Second, we used surrogate measures for our case definitions, rather than a ''hard'' classification scheme based on a case-control study design. Third, the difference in the prevalence of elevated titres among non-Hispanic whites, Mexican-Americans, and non-Hispanic blacks was not consistent across all titres, and one titre (to *M. micros*) in fact showed the opposite trend. Lastly, establishing cut-offs specific to ethnic groups could be biased because it could represent cultural effects or an influence of lower levels of acculturation, thus requiring adjustments for the control of confounding due to behavioural characteristics.

This study has some limitations that should be considered when interpreting estimates of elevated antibody status to oral bacteria. Because persons could refuse to participate in the physical examination, including phlebotomy, there is the potential for non-response bias to adversely affect the calculation of prevalence estimates. However, the use of the sample weights for examinee data includes an adjustment factor to reduce the effect of non-response bias.

Determining ranges of normal *versus* elevated values for laboratory tests is often performed by selecting a cut-off point at two or three standard deviations above or below the mean, especially for ELISA-based assays. Alternatively, cutoff points can be defined by using pre-determined sensitivity or specificity targets. For the current analyses, we selected the 90th percentile titre level in individuals without periodontal disease, defined by two widely accepted but differing definitions of periodontitis for use in epidemiologic studies, to emphasize high specificity for epidemiologic applications instead of a threshold for diagnostic purposes.

Because the composition of the NHANES III surplus sera database included a sizeable edentulous cohort

Table 5. Association* between elevated antibody titres and key periodontitis risk factors for dentate adults: United States, 1988–1994

Titres	OR (CI)						
	age group		race/ethnicity		gender	education	
	70 years/older	55–69 years	Mexican-Americans	NH-blacks	male	not finished HS	finished HS
A. actinomycetemcomitans Mix[1]				1.7 (1.4, 2.2)			
A. actinomycetemcomitans 29523		1.4 (1.1, 2.0)		1.4 (1.1, 1.8)			
A. actinomycetemcomitans Y4				1.8 (1.4, 2.3)			
P. gingivalis Mix[2]	1.9 (1.4, 2.6)		2.1 (1.5, 2.9)	3.0 (2.4, 3.7)		1.5 (1.1, 2.1)	
T. forsythia				1.4 (1.1, 1.9)			
T. denticola				1.4 (1.1, 2.0)			
C. rectus			1.6 (1.1, 2.5)	2.1 (1.6, 2.8)	1.5 (1.2, 2.0)		
E. corrodens				1.6 (1.1, 2.2)			
E. nodatum	0.6 (0.4, 0.8)					0.6 (0.4, 0.8)	
P. intermedia			1.5 (1.1, 2.2)	2.8 (2.1, 3.6)			
P. nigrescens				2.0 (1.6, 2.5)	1.5 (1.1, 2.0)		
P. melaninogenica					1.5 (1.1, 1.9)		
F. nucleatum					1.5 (1.1, 1.9)		
M. micros							
S. noxia				1.4 (1.1, 1.8)			
C. ochracea							
S. intermedius							
S. oralis				1.6 (1.2, 2.0)			
S. mutans				1.7 (1.3, 2.2)			
V. parvula	1.5 (1.1, 2.1)		0.7 (0.5, 0.9)				
A. naeslundii					0.6 (0.4, 0.9)		

Titres	OR (CI)				
	smoking status		diabetes	dental status	
	current	former	yes	1–9 Teeth	10–19 Teeth
A. actinomycetemcomitans Mix[1]					
A. actinomycetemcomitans 29523		1.5 (1.1, 1.9)	0.6 (0.4, 0.9)		
A. actinomycetemcomitans Y4			0.6 (0.4, 0.9)		
P. gingivalis Mix[2]	0.6 (0.5, 0.9)			0.7 (0.5, 0.9)	
T. forsythia					
T. denticola					
C. rectus	0.5 (0.3, 0.8)			0.4 (0.3, 0.6)	
E. corrodens					
E. nodatum	0.6 (0.4, 0.9)				0.6 (0.4, 0.9)
P. intermedia					
P. nigrescens	0.6 (0.4, 0.9)				
P. melaninogenica	0.6 (0.4, 0.9)				
F. nucleatum					
M. micros	2.2 (1.5, 3.3)	1.6 (1.1, 2.2)			
S. noxia					
C. ochracea					
S. intermedius					1.5 (1.09, 2.08)
S. oralis					
S. mutans					
V. parvula	0.5 (0.4, 0.7)				
A. naeslundii	0.6 (0.4, 0.9)				

Source: third National Health and Nutrition Examination Survey (NHANES III).

Reference groups: Age 40–54 years, non-Hispanic whites, female gender, some college education, never smokers, not diagnosed with diabetes, having 20 or more teeth.

Mix[1]: Titre to a mixed suspension of *A. actinomycetemcomitans* ATCC strains #43718, #29523, and #33384 (Y4).

Mix[2]: Titre to a mixed suspension of *P. gingivalis* ATCC strains #33277 and #53978.

*Odds ratio (OR) and 95% Confidence intervals (CI).

(1588 individuals), we were able to compare the distribution of high antibody titres in edentulous and dentate individuals. Our data clearly demonstrated that the prevalence of elevated titres was significantly lower among the edentulous participants, for 11 out of the 19 investigated IgG responses (Table 3). The highest difference was noted for *P. gingivalis* titres, with the prevalence of edentulous high responders being approximately one fourth of

Table 6. Association* between elevated antibody titres and key periodontitis risk factors for edentulous adults: United States, 1988–1994

Titres	OR (CI)						
	age group		race/ethnicity		gender	education	
	70 years/ older	55–69 years	Mexican-Americans	NH-blacks	male	not finished HS	finished HS
A. actinomycetemcomitans Mix[1]				2.3 (1.3, 3.9)		3.4 (1.2, 9.3)	
A. actinomycetemcomitans 29523			3.2 (1.6, 6.7)	2.2 (1.3, 3.6)			
A. actinomycetemcomitans Y4			3.2 (1.7, 6.0)	2.4 (1.5, 3.9)			
P. gingivalis Mix[2]				2.8 (1.5, 5.0)	2.3 (1.3, 4.4)		
T. forsythia				2.6 (1.4, 4.9)			
T. denticola							
C. rectus				3.9 (1.9, 8.0)			
E. corrodens							
E. nodatum							
P. intermedia			4.7 (2.3, 9.4)	3.9 (2.2, 6.8)			
P. nigrescens							
P. melaninogenica							
F. nucleatum					1.8 (1.1, 3.2)		
M. micros							
S. noxia							
C. ochracea				3.1 (1.7, 5.5)			
S. intermedius							
S. oralis				1.8 (1.2, 2.8)			
S. mutans			2.2 (1.2, 4.3)	2.1 (1.3, 3.2)			
V. parvula				1.7 (1.1, 2.5)			
A. naeslundii							

Titres	OR (CI)		
	smoking status		diabetes
	current	former	yes
A. actinomycetemcomitans Mix[1]			
A. actinomycetemcomitans 29523			
A. actinomycetemcomitans Y4		2.0 (1.1, 3.7)	
P. gingivalis Mix[2]			
T. forsythia			
T. denticola			
C. rectus			
E. corrodens	2.3 (1.4, 4.0)		
E. nodatum			
P. intermedia			
P. nigrescens			
P. melaninogenica			
F. nucleatum			
M. micros	3.6 (1.5, 8.9)	2.5 (1.3, 5.0)	
S. noxia			
C. ochracea			
S. intermedius			
S. oralis		1.9 (1.1, 3.4)	
S. mutans			
V. parvula			
A. naeslundii			

Source: third National Health and Nutrition Examination Survey (NHANES III).
Reference groups: Age 40–54 years, non-Hispanic whites, female gender, some college education, never smokers, not diagnosed with diabetes.
Mix[1]: Titre to a mixed suspension of *A. actinomycetemcomitans* ATCC strains #43718, #29523, and #33384 (Y4).
Mix[2]: Titre to a mixed suspension of *P. gingivalis* ATCC strains #33277 and #53978.
*Odds Ratio (OR) and 95% Confidence Intervals (CI).

that of dentate high responders (3% *versus* 11.5%). For several other titres (e.g., to *A. actinomycetemcomitans*, *T. forsythia*, *T. denticola*, *C. rectus*, and *P. intermedia*) the prevalence of edentulous high responders amounted to 40–60% of that observed in dentate individuals. Similarly, the distribution of elevated titres according to number of

teeth present generally corroborated the patterns observed in the edentulous/dentate comparisons, with the majority of the titres showing a lower prevalence of high responders among the individuals having one to nine teeth when compared with those having 20 teeth or more.

Interestingly, there was no evidence of a clear dose–response association between number of remaining teeth and the prevalence of elevated titres, as the intermediate category (i.e., individuals with 10–19 teeth present) showed the highest prevalence of high responders for a number of titres (*A. actinomycetemcomitans* Y4, *S. intermedius*) and the lowest prevalence for others (*E. nodatum*, *A. naeslundii*). Thus, the association of high antibody responses and remaining teeth seems to be quite complex: edentulous participants who are obviously not currently exposed to a periodontal infectious challenge are less likely to have retained high antibody responses, although time since tooth clearance – which has not been accounted for in our analyses – is conceivably an important determinant of their titre levels. On the other hand, the prevalence of elevated titres among individuals with different numbers of remaining teeth is also dependent on their current periodontal status (Takahashi et al. 2001, Craig et al. 2002, Furuichi et al. 2003), and the latter was found to be clearly inferior in participants with fewer remaining teeth (Table S1). As shown in Table 2, the prevalence of elevated *P. gingivalis* titres was approximately twice as high among individuals with periodontitis than among periodontal healthy participants, which corroborates earlier observations by us (Papapanou et al. 2001) and other investigators (Pussinen et al. 2002).

The above effects of periodontal status and number of teeth present on systemic antibody responses may partly underlie the associations presented in Tables 5 and 6 that describe the effects of age, race/ethnicity, gender, education and diabetes. A number of studies in the literature have suggested an increase of systemic antibody titres to periodontal bacteria with age (Mouton et al. 1981, De Nardin et al. 1991, Craig et al. 2003), and these observations were also corroborated by data in ageing non-human primates (Ebersole et al. 2008b). In contrast, in an earlier case-control study of northern Europeans, a general decline in antibody titres over the age of 55 years was observed (Papapanou et al. 2000), while a study assessing IgG serum antibody levels to *P. gingivalis* fimbiae in a rural cohort in Japan (Furuichi et al. 2001) reported no associations between age and titre levels. In the present cohort, the prevalence of five elevated titres was found to differ with respect to age, but there was no consistent directional effect. Thus, the prevalence of elevated antibody titres to *P. gingivalis* and *V. parvula* were highest in the oldest age cohort, while the prevalence of high responders to *A. actinomycetemcomitans* 29523 and Y4 peaked in the intermediate age group. In contrast, the prevalence of high responders to *E. nodatum* and *A. naeslundii* was highest among the youngest study participants.

Our observation of elevated antibody titres to several periodontal species among Hispanics and non-Hispanic blacks are in general agreement with the literature (Albandar et al. 2002, Craig et al. 2002) although high titres to a single species (*M. micros*) were found to be higher in non-Hispanic whites rather than non-Hispanic blacks in our cohort. Contrary to observations in non-human primates where female animals were found to display higher IgG responses than males (Ebersole et al. 2008c), our data showed higher prevalence of elevated titres in males than females for six periodontal species (Table 6). Individuals with low educational attainment were more frequently high responders to *A. actinomycetemcomitans*, *P. gingivalis*, and *P. intermedia* but less frequently to *E. nodatum*, *M. micros*, and *A. naeslundii*. Only the prevalence of elevated titres to *P. gingivalis* was higher in individuals with diagnosed diabetes than in diabetes-free individuals. The diabetes-related association data are not readily comparable with earlier studies that investigated IgG titres in diabetic subjects with exclusively type 1 (Sims et al. 2002, Lalla et al. 2006) or type 2 diabetes (Ebersole et al. 2008a) as an exact diagnosis on the type of diabetes was not available in the present analysis. Lastly, our data are in general agreement with the notion that smoking results in a suppressed immunoglobulin production (Califano et al. 1997, Mooney et al. 2001, Al-Ghamdi & Anil 2007), as the prevalence of high responders to nine periodontal species were significantly lower in current smokers when compared with never smokers (Table 4), eight of which remained significant in the adjusted analyses in dentate participants (Table 5). It remains unclear why responses to *M. micros* showed the opposite trend (Tables 4 and 5), as also did a few titres in edentulous patients (Table 6).

Although our analyses identified several significant determinants of the systemic antibody response to components of the dental plaque biofilm, we acknowledge that additional potentially important sources of variance were not included in our analyses. As documented in a recent study of older community dwelling adults (Singer et al. 2009), systemic oxidative stress, expressed through serum 8-isoprostane levels, was significantly associated with a generalized decrease of serum IgG responses to periodontal bacteria, and was a stronger predictor of these responses than periodontal status, age and smoking. Nevertheless, an important analytical strength of using NHANES data is the ability to characterize the prevalence of diseases and conditions in the United States. With this report, we have presented the distribution of elevated serum IgG responses to periodontal bacteria in both edentulous and dentate adults aged 40 years and older in a nationally representative sample and have demonstrated its variability across a number of demographic-, behavioural-, and health-related factors in this population.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Periodontal status, based on two alternative disease definitions, by selected demographic and health characteristics for adults aged 40 years and older[#]: United States, 1988–1994.

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Clinical Relevance

Scientific rationale for study: Periodontitis is a mixed bacterial infection, and subgingival bacterial colonization elicits a systemic antibody response. We examined the effects of selected demographic, behavioural and oral- and general health-related characteristics on the level of serum antibodies in a nationally representative sample of US adults.

Principal findings: Edentulous participants demonstrated significantly lower serum antibody levels to multiple species. Periodontal status, age, race/ethnicity, educational attainment, and smoking status also influenced the systemic antibody response.

Practical implications: The intensity of the systemic antibody responses to periodontal bacteria is determined by the complex interplay of background and behavioural factors, and oral- and general health-related characteristics.