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Periodontal pathogen carriage, rather than periodontitis, determines the serum antibody levels

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Abstract

Aim: We investigated in a nationally representative sample, how periodontitis modifies the association between the carriage of periodontal pathogens and serology.

Materials and Methods: The population comprised 1586 dentate subjects who participated in an interview, clinical and radiological oral health examination, and saliva collection. Serum immunoglobulin A (IgA)- and IgG-class antibody levels against *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* and their salivary occurrence were determined in the whole population. The quantity of the pathogens was measured in a subpopulation.

Results: In the univariate analyses, the corresponding antibody levels were higher in the pathogen carriers compared with the non-carriers, and clearly higher in the carriers with periodontal pockets compared with the carriers without. In the multi-variate analyses, however, all antibody levels associated strongly with age ($p < 0.001$) and the carriage of the corresponding pathogen ($p < 0.001$), but only weakly with the presence or number of teeth with periodontal pockets. In the subpopulation, the antibody levels and the numbers of corresponding bacteria in saliva had a positive association, which was not affected by the disease.

Conclusions: The carriage of *A. actinomycetemcomitans* and *P. gingivalis* is the strongest determinant of the systemic antibody response to these pathogens, and the extent of periodontitis has at most a modest modifying effect.

Key words: antibody; infection; inflammation; periodontal pathogens; periodontitis; saliva; serology

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Periodontitis is a persistent polymicrobial infection, which leads to chronic inflammation in the tooth-supporting tissues. It is one of the most common infections worldwide; the National Health 2000 survey with clinical oral examination revealed that teeth with $\geqslant 4$ mm periodontal pockets are found in 64% and those with $\geqslant 6$ mm pockets in 21% of adult Finns (Suominen-Taipale et al. 2008). Because several epidemiological and clinical studies have shown that periodontitis may threaten general health by increasing the risk of cardiovascular diseases, preterm birth, diabetes mellitus, and pulmonary diseases (Scannapieco et al. 2010), the need for developing useful systemic markers of periodontitis has become apparent.

Conflict of interest and source of funding statement

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Aggregatibacter actinomycetemcomitans and *Porphyromonas gingivalis* are two major pathogens aetiologically linked to periodontal disease. They both are Gram-negative, anaerobic, and serologically heterogeneous species. They are considered residents of the oral microbiota and frequently found in low numbers in periodontally healthy subjects also (Hyvärinen et al. 2009). The occurrence of periodontal pathogens in saliva is very common; 20.0% and 35.4% of subjects in the Health 2000 survey were carriers of *A. actinomycetemcomitans* and *P. gingivalis*,

respectively, and 88.2% of the subjects were positive for at least one of the six pathogens examined (Könönen et al. 2007).

Serum antibody levels against infecting agents, i.e., bacteria, viruses, and fungi, are frequently used as diagnostic tools in clinical laboratories and epidemiological studies. Such an assay would be particularly useful for periodontitis, which can otherwise be diagnosed only by dental professionals in time-consuming and expensive clinical examinations not always suitable for large-scale studies. However, there are only a few larger studies combining clinical, bacterial, and serological data (Papapanou et al. 2000) that would explore the role of both periodontitis and the occurrence of periodontal pathogens in the systemic antibody response. Therefore, as a representative of such, we investigated the association between the carriage of *A. actinomycetemcomitans* and *P. gingivalis* and their serum antibody levels, and furthermore, whether this association is modified by clinically determined periodontitis.

Materials and Methods

Survey

Pathogen carriage was studied in a subpopulation of the population-based ''Health 2000'' Health Examination Survey (http://www.ktl.fi/health2000/index.uk.html). Data were collected by questionnaires, interviews, and health examinations, including laboratory tests. The study protocol was approved by the local ethics committees, and the participants signed an informed consent.

A total of 1799 serum and salivary samples were collected from subjects, who participated in both the interviews and the clinical oral health examination. Out of these, 1292 samples were collected in the southern Finland district during the base study in 2000–2001 (Könönen et al. 2007) and 507 during a supplementary study conducted in university hospitals in 2001–2002. The present study comprises data on all dentate subjects whose periodontal health was determined with a total number of 1586.

Laboratory determinations

Paraffin-stimulated whole saliva was aliquoted and frozen in carbonic ice immediately after expectoration. The specimens were stored at -70°C until bacterial detection. Bacterial DNA was extracted from saliva with cation-chelating resin (Chelex 100; Bio-Rad Laboratories, CA, USA), and *A. actinomycetemcomitans* and *P. gingivalis* were detected by multiplex polymerase chain reaction (PCR) using conserved species-specific primers (Tran & Rudney 1999, Könönen et al. 2007, Paju et al. 2009). In a subpopulation of 165 subjects, quantitative pathogen data were available from our previous study (Hyvärinen et al. 2009).

Serum immunoglobulin A (IgA)- and IgG-class antibodies to *A. actinomycetemcomitans* and *P. gingivalis* were determined from frozen sera (-20°C) by multi-serotype enzyme-linked immunosorbent assay (ELISA) (Pussinen et al. 2002). As designed for large studies, two selected dilutions of each serum in duplicates were used and the results consisting of mean absorbances from four wells were calculated as continuous variables (Pussinen et al. 2003). The dilutions (v:v) were 1:1500 and 1:3000 for *A. actinomycetemcomitans* IgG, and 1:100 and 1:200 for *A. actinomycetemcomitans* IgA and *P. gingivalis* IgG and IgA (Pussinen et al. 2003). After the whole material was analysed, the results were normalized according to coefficients obtained from reference serum samples applied on each plate. The inter-assay coefficients for variation ($n = 92$) were 5.9% and 5.5% for *A. actinomycetemcomitans* and 4.9% and 4.7% for *P. gingivalis* IgA and IgG, respectively.

Oral health examination and panoramic tomographs

The clinical oral examination performed by specially trained dentists has been described in detail (Könönen et al. 2007, Suominen-Taipale et al. 2008, Paju et al. 2009). The registered parameters included the number of teeth (all teeth and tooth remnants) and the number of periodontally diseased teeth with probing pocket depths of $\geqslant 4$ and $\geqslant 6$ mm. Periodontal pocket depth was measured on four surfaces of each tooth apart from the third molars in the following order: distal angle and midpoint on the buccal side, midpoint on the lingual side, and mesial angle. Only the pocket depth of the deepest site on each tooth was recorded. The number of diseased teeth with angular bone defects extending at least to the middle third of the root was registered from the panoramic tomographs.

Data analyses

We calculated the mean serum IgA- and IgG-class antibody levels to *A. actinomycetemcomitans* and *P. gingivalis* according to the pathogen carriages in saliva and oral health status. Owing to highly skewed distributions of the serum antibody levels, model assumptions (normality of residuals and constant variances) for linear regression were not fulfilled. We therefore used Poisson's regression models to study the associations between the serum antibody levels and pathogen carriage and periodontal health. Separate models including the variables describing periodontal health (the number of teeth with probing pocket depth $\geqslant 4$ or $\geqslant 6$ mm, or with angular bone defects as continuous variables) were fitted and further adjusted for pathogen carriage. In addition, all the associations were adjusted for age (continuous variable), gender, education level, smoking habit, and examination time (base or supplementary study). The number of teeth was used as an offset variable. Smoking was categorized into four classes to distinguish never, former, occasional, and daily smokers. We studied a possible effect modification of periodontal health by adding the interaction terms between pathogen carriage and the number of teeth with periodontal pockets $\geqslant 4$ mm categorized into (i) none, (ii) 1–6, and (iii) 7 or more, one by one in the fully adjusted regression models for each serum antibody level. A weak interaction was detected between presence of *P. gingivalis* and number of teeth with periodontal pockets $\geqslant 4$ mm in IgA- ($p = 0.22$) and IgG- ($p = 0.12$) antibody levels to *P. gingivalis*. Therefore, stratified analyses by the categorized number of teeth with periodontal pockets $\geqslant 4$ were conducted.

The association between serum antibody levels and quantitative PCR (qPCR) results from the subpopulation ($n = 165$) (Hyvärinen et al. 2009) was analysed by multiple linear regression models, adjusted stepwise for the age, gender, smoking (smokers *versus* non-smokers), and periodontal status (healthy *versus* advanced periodontitis).

Results

The characteristics of the dentate subjects are presented in Table 1. The mean (SD) age of the subjects was 50.4 (12.3) years and their mean number of teeth

Table 1. Characteristics of the subjects ($n = 1586$)

	n	%
Women	863	54.1
Age group		
30–39	359	22.6
40–49	407	25.7
50–59	471	29.7
60–69	227	14.3
70+	122	7.7
Level of education		
Basic	434	27.5
Secondary	497	31.4
Higher	650	41.1
Smoking history		
Never	775	49.0
Former	356	22.5
Occasional	84	5.3
Daily	366	23.2
Number of teeth		
25–32	1046	66.0
20–24	259	16.3
10–19	149	9.4
1–9	132	8.3
Number of teeth with ⩾4 mm pockets		
0	383	24.2
1–2	313	19.7
3–6	397	25.0
7+	493	31.1
Number of teeth with ⩾6 mm pockets		
0	1224	77.2
1	144	9.1
2–4	125	7.9
5+	93	5.9
Number of teeth with angular bone defects*		
0	1446	91.2
1+	140	8.8
Presence of pathogen in saliva		
Aggregatibacter actinomycetemcomitans	337	21.3
Porphyromonas gingivalis	616	61.2

*Extending at least to the middle third of the root in the radiograph.

Table 2. Mean (SD) antibody levels according to the pathogen carriage and periodontal status

	n	Mean (SD)			
		Aa IgA (EU)	Aa IgG (EU)	Pg IgA (EU)	Pg IgG (EU)
All	1586	1.92 (1.64)	3.34 (2.10)	2.33 (2.98)	6.41 (3.94)
Pathogen in saliva					
Aa in saliva					
Yes	337	3.24 (2.12)	5.58 (2.15)	2.46 (3.09)	6.69 (4.20)
No	1249	1.56 (1.26)	2.74 (1.62)	2.29 (2.95)	6.34 (3.87)
Pg in saliva					
Yes	616	2.02 (1.62)	3.51 (2.00)	4.29 (3.73)	9.76 (3.96)
No	970	1.85 (1.65)	3.24 (2.15)	1.08 (1.32)	4.29 (1.96)
Periodontal status					
Clinical examination					
Number of teeth with pockets ⩾4 mm					
0	383	1.61 (1.27)	3.14 (1.89)	1.62 (2.09)	5.21 (3.12)
1–2	313	1.90 (1.71)	3.30 (2.07)	1.85 (2.45)	5.72 (3.35)
3–6	397	1.98 (1.66)	3.43 (2.12)	2.49 (3.03)	6.56 (3.93)
7–	493	2.13 (1.78)	3.46 (2.24)	3.04 (3.61)	7.67 (4.48)
Number of teeth with pockets ⩾6 mm					
0	1224	1.80 (1.50)	3.26 (2.08)	1.94 (2.58)	5.76 (3.51)
1	144	2.33 (2.03)	3.76 (2.15)	3.27 (3.34)	8.19 (4.09)
2–4	125	2.29 (2.00)	3.54 (2.08)	3.64 (3.75)	8.42 (4.87)
5–	93	2.40 (1.88)	3.49 (2.23)	4.17 (4.37)	9.63 (4.44)
Radiographic findings					
Angular bone defects					
Yes	140	2.30 (1.92)	3.54 (2.24)	4.00 (4.16)	9.15 (4.48)
No	1446	1.88 (1.60)	3.32 (2.08)	2.16 (2.79)	6.15 (3.79)

Aa, *Aggregatibacter actinomycetemcomitans*; EU, ELISA units; Ig, immunoglobulin; Pg, *Porphyromonas gingivalis*.

24.0 (7.0). Oral health examination revealed that the subjects had on average 5.4 (6.1) and 0.8 (2.5) teeth with periodontal pockets exceeding ⩾4 and ⩾6 mm, respectively. The mean number of teeth with angular bone defects was 0.2 (0.7).

The IgA- and IgG-class antibody levels to the examined pathogens were clearly higher in the pathogen carriers compared with the non-carriers (Table 2). The antibody levels related to the number of teeth with deepened pockets in a dose-dependent manner and were higher among those having angular bone defects. The association between the number of teeth with ⩾6 mm pockets and the antibody levels was more obvious for *P. gingivalis* than for *A. actinomycetemcomitans* (Table 2). When the antibody levels were further analysed for the corresponding pathogen carriage, the association was seen only in *P. gingivalis*-positive subjects (Fig. 1). The cut-off limits determined earlier for seronegative and seropositive values for both IgG- and IgA-antibody classes, 5.0 and 2.0 EU ELISA, respectively (Pussinen et al. 2002), distinguished clearly the pathogen carriers from the non-carriers (Fig. 1).

The associations of serum antibody levels with the pathogen carriage, and clinical and radiographic findings were analysed by multi-variate regression models (Table 3). The highest rate ratios were found between the pathogen carriage and the antibody levels of the corresponding pathogen, as expected. Weak or borderline associations were observed only between the antibody levels against *P. gingivalis* and ⩾6 mm pocket teeth or radiographic findings. In the analysis stratified according to the number of teeth with periodontal pockets ⩾4 mm, the association between the antibody levels to *A. actinomycetemcomitans* and its carriage was only weakly dependent on the presence or extent of the disease (Table 4). On the contrary, the modifying effect of the disease on the association of *P. gingivalis* antibody levels and the carriage was stronger, especially in the case of IgA-class antibodies.

Quantitative PCR results on salivary *A. actinomycetemcomitans* and *P. gingivalis* levels were available from a subpopulation of 165 subjects. Both pathogen quantities had a significant ($p < 0.001$) correlation with the corresponding serum IgA- and IgG-antibody levels, the *r*-values being 0.376 and 0.373 for *A. actinomycetemcomitans*, and 0.551 and 0.562 for *P. gingivalis*, respectively (Table 5). These correlations were not notably affected by adjustments for the age, gender, smoking, or periodontal status.

Discussion

In this large population-based sample, we show that the main determinant of the systemic antibody response to two major periodontal pathogens, *A. actinomycetemcomitans* and *P. gingivalis*, is the oral carriage of the pathogen. The presence or extent of periodontitis has, at the most, a modest modifying effect on the antibody levels. Using the present multi-serotype ELISA, seropositive subjects can be considered pathogen carriers and the serum antibody levels

associate strongly with the corresponding bacterial amounts in saliva.

Owing to the methodological challenges associated with the multi-bacterial nature of periodontitis, there have been only a few attempts with different approaches to develop a serological diagnostic tool suitable for large-scale studies of periodontitis (Sakellari et al. 1997, Papapanou et al. 2000, 2001, Pussinen et al. 2002). Papapanou and colleagues have determined by the checkerboard immunoblotting the serum IgG-class antibody levels to 19 periodontal bacterial species, including established pathogens, putative pathogens, and non-pathogenic species, and explored whether they could serve as surrogate markers of clinical periodontal status. Patients with periodontitis displayed antibody patterns, which were distinct from subjects with no periodontal breakdown (Papapanou et al. 2004). In a large population of US adults who participated in the third National Health and Nutrition Examination Survey, high *P. gingivalis* titres were consistently associated with periodontitis, whereas high *Eubacterium nodatum* titres were associated with periodontal health (Dye et al. 2009). Furthermore, the authors concluded that oral and general health-related characteristics, including dental status and diabetes, are strong determinants of the systemic antibody responses (Vlachojannis et al. 2010). In addition to the method used to determine the antibody levels, the present study differs from the previous ones (Dye et al. 2009, Vlachojannis et al. 2010) by the definitions of periodontitis. While we have used mainly the number of teeth with deepened periodontal pockets, either exceeding 4 or 6 mm, in five categories, the other investigators have used two (Vlachojannis et al. 2010) or three (Dye et al. 2009) alternative definitions for pathological periodontal status based on inter-proximal attachment loss and probing pocket depth. Both studies found high *P. gingivalis*

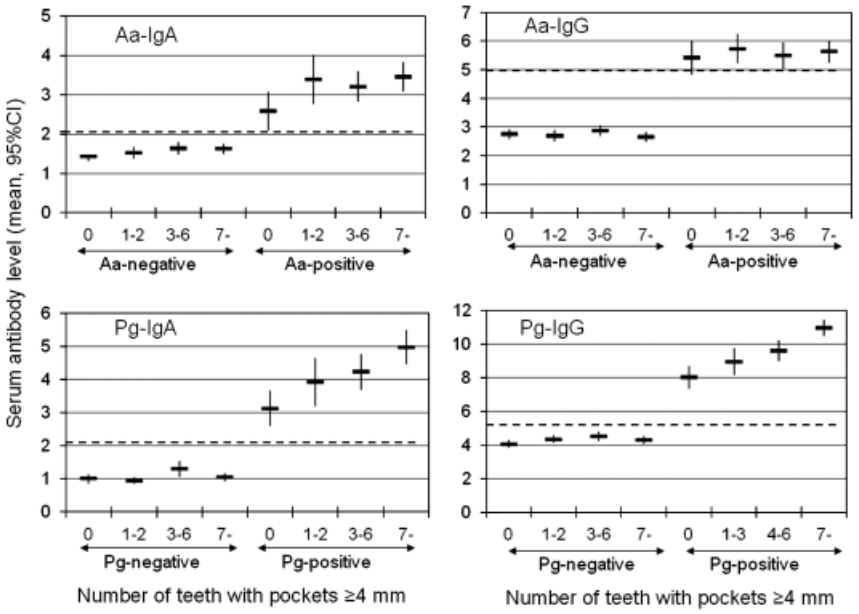

Fig. 1. Mean antibody levels according to the pathogen carriage and number of teeth with periodontal pockets. Mean (95% confidence interval) serum antibody levels to *Aggregatibacter actinomycetemcomitans* (Aa) and *Porphyromonas gingivalis* (Pg) were determined according to the pathogen carriage in saliva by species-specific polymerase chain reaction and the number of teeth with probing pocket depth ⩾4 mm as detected in the clinical examination. The cut-off limits for seropositive values (Pussinen et al. 2002) are depicted by dashed lines.

Table 3. Associations of the serum antibody levels with the pathogen carriage and three periodontal parameters

Antibody class	Rate ratio (95% confidence interval)								
model*	saliva Aa		saliva Pg		pockets ⩾4 mm		pockets ⩾6 mm		angular bone defects
Aa IgA									
Model 1	–		–		1.00 (0.99–1.01)	or	1.01 (0.99–1.03)	or	1.02 (0.97–1.07)
Model 2	2.00 (1.83–2.18)	and	0.98 (0.90–1.07)		–		–		–
Model 3	2.02 (1.86–2.21)	and	1.00 (0.92–1.10)	and	0.99 (0.98–1.00)	or	1.00 (0.99–1.02)	or	1.00 (0.95–1.06)
Aa IgG									
Model 1	–		–		0.99 (0.99–1.00)	or	1.00 (0.98–1.01)	or	0.98 (0.94–1.02)
Model 2	1.94 (1.82–2.06)	and	0.99 (0.93–1.06)		–		–		–
Model 3	1.97 (1.85–2.10)	and	1.02 (0.95–1.09)	and	0.99 (0.98–0.99)	or	0.99 (0.98–1.00)	or	0.96 (0.93–1.00)
Pg IgA									
Model 1	–		–		1.01 (1.00–1.02)	or	1.04 (1.02–1.05)	or	1.15 (1.08–1.23)
Model 2	0.93 (0.82–1.07)	and	3.60 (3.20–4.04)		–		–		–
Model 3	0.95 (0.84–1.08)	and	3.69 (3.28–4.15)	and	0.99 (0.98–1.00)	or	1.01 (1.00–1.02)	or	1.08 (1.02–1.14)
Pg IgG									
Model 1	–		–		1.00 (0.99–1.01)	or	1.03 (1.02–1.04)	or	1.10 (1.07–1.14)
Model 2	0.96 (0.90–1.01)	and	2.16 (2.04–2.28)		–		–		–
Model 3	0.97 (0.91–1.03)	and	2.20 (2.09–2.33)	and	0.99 (0.99–1.00)	or	1.01 (1.00–1.02)	or	1.06 (1.03–1.08)

Number of teeth with pockets ⩾4 or ⩾6 mm or angular bone defects added as continuous variables, the presence of Aa and Pg in saliva as categorical variables.

*Adjusted for the age (continuous), gender, education level, smoking, examination time (base or supplementary study), and number of teeth (offset-variable).

Aa, *Aggregatibacter actinomycetemcomitans*; Pg, *Porphyromonas gingivalis*.

Table 4. Associations of the serum antibody levels with the pathogen carriage stratified by the number of teeth with periodontal pockets

Antibody class	Rate ratio (95% confidence interval)								
	number of teeth with deepened (⩾4 mm) periodontal pockets								
model*	none ($n = 383$)			1–6 ($n = 706$)			7 or more ($n = 492$)		
	saliva Aa		saliva Pg	saliva Aa		saliva Pg	saliva Aa		saliva Pg
Aa IgA									
Model 1	1.74 (1.40–2.19)		–	2.12 (1.86–2.41)		–	2.11 (1.84–2.42)		–
Model 2	–		NA	–		NA	–		NA
Model 3	1.75 (1.39–2.20)	and	NA	2.12 (1.86–2.42)	and	NA	2.11 (1.84–2.41)	and	NA
Aa IgG									
Model 1	1.77 (1.51–2.08)		–	1.97 (1.78–2.17)		–	2.09 (1.90–2.30)		–
Model 2	–		NA	–		NA	–		NA
Model 3	1.77 (1.51–2.08)	and	NA	1.97 (1.78–2.17)	and	NA	2.09 (1.90–2.30)	and	NA
Pg IgA									
Model 1	NA		–	NA		–	NA		–
Model 2	–		3.05 (2.41–3.86)	–		3.51 (2.91–4.24)	–		4.32 (3.62–5.15)
Model 3	NA	and	3.05 (2.42–3.86)	NA	and	3.52 (2.91–4.25)	NA	and	4.32 (3.63–5.15)
Pg IgG									
Model 1	NA		–	NA		–	NA		–
Model 2	–		2.01 (1.76–2.29)	–		2.07 (1.90–2.26)	–		2.47 (2.27–2.69)
Model 3	NA	and	2.02 (1.77–2.30)	NA	and	2.07 (1.90–2.26)	NA	and	2.47 (2.27–2.69)

*Adjusted for the age (continuous), gender, education level, smoking, examination time (base or supplementary study), and number of teeth (offset-variable). The presence of Aa and/or Pg in saliva is added as categorical variables.
Aa, *Aggregatibacter actinomycetemcomitans*; NA, not associated; Pg, *Porphyromonas gingivalis*.

Table 5. Association of salivary pathogen quantities with the serum antibody levels by linear regression analysis

	Saliva Aa (GE/ml) bivariate	Model 1 age	Model 2 +gender	Model 3 +smoking	Model 4 +periodontitis
Serum Aa-IgA (EU)	R 0.376	R 0.378	R 0.378	R 0.385	R 0.398
	R^2 0.141	R^2 0.143	R^2 0.143	R^2 0.148	R^2 0.159
	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
Serum Aa-IgG (EU)	R 0.373	R 0.373	R 0.381	R 0.421	R 0.428
	R^2 0.139	R^2 0.139	R^2 0.145	R^2 0.177	R^2 0.183
	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
	Saliva Pg (GE/ml) bivariate	Model 1 age	Model 2 +gender	Model 3 +smoking	Model 4 +periodontitis
Serum Pg-IgA (EU)	R 0.551	R 0.563	R 0.563	R 0.571	R 0.574
	R^2 0.303	R^2 0.317	R^2 0.317	R^2 0.327	R^2 0.329
	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
Serum Pg-IgG (EU)	R 0.562	R 0.568	R 0.580	R 0.582	R 0.583
	R^2 0.316	R^2 0.323	R^2 0.336	R^2 0.339	R^2 0.340
	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$

Model 1: adjusted for the age (continuous); Model 2: adjusted for the age and gender; Model 3: adjusted for the age, gender, and smoking status (smoker *versus* non-smoker); Model 4: adjusted for the age, gender, smoking status, and presence of periodontitis (advanced periodontitis *versus* periodontally healthy).
Aa, *Aggregatibacter actinomycetemcomitans*; EU, ELISA units; GE, genome equivalents; Pg, *Porphyromonas gingivalis*;.

IgG-antibody titres to be associated with periodontitis. Also in the present study the correlation between the number of periodontal pockets and the antibody levels to *P. gingivalis* was stronger than that for *A. actinomycetemcomitans*. These cross-sectional studies, however, do not explain when the IgG-class antibody response has evolved. IgA response, one the other hand, arises from a continuous exposure, because these antibodies have a short half-life.

Although a number of multiple positive periodontal pathogens, rather than a single periopathic species, are associated with the presence of periodontitis (Paju et al. 2009), our group has successfully used the antibody levels to two major periodontal pathogens, *A. actinomycetemcomitans* and *P. gingivalis*, as a surrogate marker of periodontitis

(Vilkuna-Rautiainen et al. 2002). The multiserotype ELISA, consisting of several strains representing different serotypes as whole-cell antigens, has the sensitivity and specificity of 71% and 90% for finding clinical periodontitis according to a relatively small case-control setting that was used to validate the assay (Pussinen et al. 2002). Because these two pathogens are frequently found in patients with periodontitis, the accuracy of the assay may as well arise from the pathogen carriage. In the present study, the antibody levels were higher in the diseased pathogen carriers compared with the healthy carriers. However, the potency of the disease to increase the antibody levels due to the systemic exposure of the pathogens via gingival bleeding and the inflamed periodontium was not as strong as expected. Furthermore, in the quantitative analyses, the occurrence of periodontitis did not have any effect on the strong association between the antibody levels and the pathogen amounts. An association between serum antibody levels to *P. gingivalis* and its subgingival colonization has been earlier found in another study (Kojima et al. 1997).

We have mainly used the serum antibody determinations to evaluate the cardiovascular disease risk associated with the systemic exposure to periodontal pathogens in populations with no dental records available (Pussinen et al. 2003, 2004, 2005, 2007a, b). Beck et al. (2005) have suggested that the quality and quantity of the bacterial exposure may be more important to systemic health than clinical measures; in their study, the clinical signs of periodontitis did not associate with prevalent coronary heart disease, whereas the systemic antibody response for several oral organisms did. On the one hand, the immunological response is certainly dependent, at least, on genetic factors, characteristics of the antigen, and the infection route. On the other hand, periodontitis is episodic in nature: Although IgG-class antibodies to periodontal pathogens are stable over time (Papapanou et al. 2004, Lakio et al. 2009) and do not necessarily respond to treatment (Darby et al. 2001), both the pathogen and antibody levels may vary according to the active and inactive disease phases. Therefore, a better diagnostic tool for periodontal disease might be a combination of both host- and microbe-derived biomarkers.

The strengths of the present study include the large, population-based sample with socioeconomically relatively homogeneous Finns. As a limitation, it can be seen that the presence and extent of periodontitis are based on the number of teeth with pocket depths exceeding 4 and 6 mm and no information on bleeding on probing is available. However, relatively shallow pockets may best reflect the whole mouth exposure to bacterial burden (Demmer et al. 2010). Therefore, we used the number of teeth with $\geqslant$4 mm pockets in the stratified analyses. This kind of measurement also had a large variation in the population providing proper group sizes for analyses.

Our results from this large population-based study with clinical, microbiological, and immunological measures indicate that the pathogen carriage is the strongest determinant of the systemic antibody response to periodontal pathogens.

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Clinical Relevance

Scientific rationale for study: Periodontitis is associated with the carriage of periodontal pathogens, which give rise to systemic antibody response. We investigated in a nationally representative sample, how clinical periodontitis modifies serum antibody levels against major periodontal pathogens.

Principal findings: In addition to age, the main determinant of the systemic antibody response to periodontal pathogens was their oral carriage, and furthermore, the bacterial numbers in saliva. The presence or extent of periodontitis had only a modest modifying effect on the antibody levels.

Practical implications: The present findings are fundamental for interpreting results on periodontitis-associated serology.