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Longitudinal stability of serum immunoglobulin G responses to periodontal bacteria

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Abstract

Background: The value of seroepidemiology in the study of periodontal infections has not been adequately explored. This study examined serum immunoglobulin (IgG) responses to periodontal bacteria in patients with periodontitis and periodontitis-free individuals over a 30-month period.

Methods: Eighty-nine patients with chronic periodontitis and 42 control subjects with no deep periodontal pockets and no or minimal attachment loss (30–72 years old, 43% men) were included. Patients were examined at baseline, after completed periodontal therapy 4 months post-baseline, and at 30 months, and controls, at baseline and 30 months. IgG antibodies to 19 periodontal species were determined by checkerboard immunoblotting.

Results: On average, patients displayed at baseline up to 800-fold higher titers than controls to all but three species. Over the 30-month period, titers remained stable at low levels in controls. In patients, periodontal conditions improved from a baseline mean probing depth of 3.6 mm, bleeding on probing of 62% and an average of 21.5 pockets of = 6 mm/person, to 2.5 mm mean pocket depth, 30% bleeding on probing, and 1.2 deep pockets, at 30 months. Over time, antibody titers showed a modest decline in patients, but remained significantly elevated at 30 months in comparison with controls. Antibody-level changes over time were not significantly different between subjects that did and did not receive adjunctive systemic antibiotics.

Conclusions: Conspicuous differences in IgG titers to periodontal bacteria exist between periodontitis patients and periodontally healthy controls. Despite successful periodontal therapy, titers remained elevated over a 30-month period, suggesting that serology may mark the history of past periodontal infection.

Key words: immunoglobulin G antibody; longitudinal; periodontal therapy

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Serology is a widely used epidemiologic tool in the study of bacterial and viral infections (Thomas & Quinn 1993, Slomka 1996, Dillner 1999, Siscovick et al. 2000), but is largely unexplored as an instrument for epidemiologic studies of periodontal diseases. Earlier studies have primarily focused on the correlation of antibody titers against specific periodontal microorganisms with periodontal disease status (Haffajee et al. 1995, Papapanou et al. 2000, Craig et al. 2001), or on their ability to serve as predictors of recurrent or refractory periodontal disease (Haffajee et al. 1988a, b, Taubman et al. 1992, Ebersole et al. 1995). To explore the potential of seroepidemiology in the study of periodontal infections, it is important to examine longitudinally both subjects with periodontitis and disease-free individuals, in order to determine the stability of such titers over time as well as to assess the effect of periodontal therapy on antibody levels. Indeed, the literature indicates that antibody levels to specific periodontal bacteria may change substantially as a result of therapy (e.g. Ebersole et al. 1985, Mooney et al. 1995). In this study, we monitored prospectively over a 30-month period serum immunoglobulin G (IgG) levels against a broad panel of periodontal bacteria in subjects treated for moderate-to-severe periodontal disease and in subjects with intact periodontal tissue support. Our results demonstrate only a modest reduction in antibody levels as a result of periodontal therapy and a conspicuous difference in titers against certain species between patients and controls at all time points.

Material and Methods

The design and methods of the study were approved by the Committee of Research Ethics, Göteborg University, Sweden. Participating subjects signed informed consent forms.

Subject sample

This report is based on the follow-up subsample of a previously described group of periodontitis patients and gender and age-matched controls (Papapanou et al. 2000, 2001). The original sample included subjects with destructive periodontitis (cases), and individuals with no or minimal loss of periodontal tissue support (controls). Cases were recruited among first time referrals for periodontal specialty care. Patients were over 20 years old, had moderate-to-advanced periodontal disease with several inflamed periodontal pockets, loss of attachment, and radiographic evidence of bone loss, and had no history of previous periodontal therapy. Control subjects displayed no sites with probing depth exceeding 5 mm with concomitant loss of clinical attachment of >1 mm. In ages below 45 years, they showed no interproximal or lingual sites with loss of clinical attachment of >3 mm. In ages over 45 years, they harbored no more than four interproximal sites with clinical attachment loss of maximally 5 mm. Cases and controls had not used antibiotics during the preceding 6-month period.

Table 1 provides a description of the follow-up sample. Out of the initially enrolled 205 subjects (132 cases and 73 controls), 131 subjects, 89 cases and 42 controls were available for the 30-month follow-up. Forty-two percent of the cases and 45% of the controls were male. The vast majority of subjects (95%) were over 35 years old.

Periodontal therapy

All patients were treated by a single periodontist (author A.M.N.). Periodontal therapy included instructions in oral hygiene, extractions of non-salvageable teeth and full-mouth scaling and root planing. Access periodontal surgery was performed in 67 patients (75.3%), at one or several quadrants depending on their individual needs. Adjunctive systemic antibiotics (tetracycline, metronidazole, or combination of amoxicillin and metronidazole, at standard dosage for a week) were prescribed to 49 patients (55.1%), all of whom were treated surgically.

Table 1. Age and gender distribution in the study sample

Age (years)	Patients		Controls		All
	men	women	men	women	
35	1	3	0	2	6
36–55	25	32	16	18	91
56	11	17	3	3	34
all	37	52	19	23	131

Clinical examination

At baseline, cases and controls were assessed with respect to plaque, bleeding on probing, probing depth and attachment levels at six sites per tooth, at all present teeth excluding third molars (Papapanou et al. 2000). Cases were re-examined 4 months after baseline (approximately 2 months after completion of periodontal therapy), and at 30 months.

Blood samples

To obtain serum samples, 10 ml of venous blood was obtained from each person by means of untreated Vacutainer™ blood collection tubes (Beckton-Dickinson, Franklin Lakes, NJ, USA). Cases were sampled at baseline, 4, and 30 months, and controls, at baseline and 30 months. The blood samples were stored at $+4^{\circ}$C overnight and thereafter centrifuged at 3000 rpm for 10 min. Serum was collected, aliquoted and stored at -70°C until further analysis.

Assessment of serum IgG antibodies to periodontal bacteria

The level of serum antibodies was assessed by means of checkerboard immunoblotting (Sakellari et al. 1997) as previously described (Papapanou et al. 2000). In brief, PBS dilutions of sonicated bacterial extracts derived from each of the 19 species listed below were brought to an optical density of 1, using a spectrophotometer (Ultrospec III, Pharmacia, Täby, Sweden) set at a wavelength of 600 nm. Extracts were prepared from *Porphyromonas gingivalis* (strain FDC381), *Porphyromonas endodontalis* (OMGS 1205), *Prevotella intermedia* (ATCC 25611), *Prevotella nigrescens* (ATCC 33563), *Prevotella melaninogenica* (OMGS 1503), *Tannerella forsythensis* (ATCC 43037), *Actinobacillus actinomycetemcomitans* (FDC Y4), *Fusobacterium nucleatum* (ATCC 10953), *Treponema denticola* (OMGS 3271), *Micromonas micros* (ATCC 33270), *Campylobacter rectus* (ATCC 33238), *Eikenella corrodens* (ATCC 23834), *Eubacterium nodatum* (OMGS 3356), *Selenomonas noxia* (OMGS 3118), *Streptococcus intermedius* (OMGS 3177), *Streptococcus oralis* (ATCC 35037), *Veillonella parvula* (ATCC 10790), *Capnocytophaga ochracea* (ATCC 27872) and *Actinomyces naeslundii* (ATCC 49340).

Protein-A standards were prepared at a concentration of 5 μg/ml. A Miniblotter™ device (Immunetics, Cambridge, MA, USA) was used to immobilize antigenic extracts and protein-A standards on nitrocellulose membranes. Serum dilutions (1/1000) from each subject and time point as well as human IgG standards (2000, 1000, 500, 333, and 167 ng/ml) were loaded perpendicular to the bacterial extracts, and were allowed to interact. After several washing steps, the membranes were incubated with Fab fragments of anti-human IgG conjugated with horseradish peroxidase. Membranes were thereafter incubated with a horseradish-peroxidase substrate (ECL Western blotting detection reagents, Amersham-Pharmacia Biotech, Täby, Sweden) and the chemiluminescent signal was assessed in a LumiImager™ Workstation (Roche–Boehringer Mannheim, Bromma, Sweden) by means of the LumiAnalyst™ software (Roche–Boehringer Mannheim) in comparison with the standard curve generated by the IgG standards, quantified in nanograms per milliliter, and exported to Excel files.

Statistical analysis

Data analysis was performed using the SAS software version 8.4 (SAS Institute Inc., Cary, NC, USA). In all analyses, the individual subject was the computational unit. Log transformations of the antibody data were performed to normalize the distribution of the raw data. In order to account for raw antibody values equal to zero the following formula was used: log antibody value = log 10 (raw antibody value+1). The statistical methods used included descriptive statistics, analysis of variance with the Tukey correction in cases of equal number of observations among groups, and general linear model test in cases of unequal numbers.

Results

Table 2 provides a summary of the clinical characteristics in cases and controls at baseline, as well as at 4 and 30 months in cases. Cases had initially fewer teeth, more sites with plaque and gingivitis, and higher mean probing depths and attachment levels compared with controls. At 30 months, plaque was reduced to 21.7%, bleeding on probing to 30.2% and there was an average 1.2 deep ($\geqslant$6 mm) pockets remaining per subject, from an average of 21.5 at baseline. Although the mean attachment level remained relatively constant during the follow-up period, there was a substantial decrease in the mean number of sites with AL$\geqslant$6 mm, from 20.5 at baseline to 14.4 at 30 months.

Fig. 1 illustrates an overview of the observed mean antibody responses in cases and controls over the entire follow-up period. The upper panel depicts responses to established periodontal pathogens such as *A. actinomycetemcomitans* and the ''red complex'' bacteria (Socransky et al. 1998). The mid-panel describes responses to several other species associated with periodontitis, and the lower panel, responses to bacteria primarily associated with gingivitis and periodontal health. Titers are presented in a logarithmic scale.

A comparison between cases and controls showed clearly that periodontitis patients exhibited conspicuously stronger responses than periodontally intact subjects against all the established periodontal pathogens at all three time points (top panel). A comparison between case and control titers to *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythensis*, and *T. denticola* at 30 months by means of a *t*-test revealed significant differences for all four titers ($p<0.001$). Furthermore, at 30 months, cases displayed higher titers than controls against *P. intermedia* ($p<0.001$), *P. nigrescens* ($p<0.001$), *M. micros* ($p<0.001$), *C. rectus* ($p<0.001$), *F. nucleatum* ($p<0.001$), *S. intermedius* ($p<0.001$), *E. nodatum* ($p=0.002$), *S. oralis* ($p=0.002$), *C. ochracea* ($p=0.002$), and *E. corrodens* ($p<0.001$). Controls displayed statistically significantly higher titers against *P. melaninogenica* ($p=0.02$), and *A. naeslundii* ($p=0.05$), while no statistically significant differences could be detected in titers to *P. endodontalis* ($p=0.1698$) and *V. parvula* ($p=0.2756$).

Detailed comparisons of antibody levels among the three occasions performed by means of analysis of variance with the Tukey correction or by a general linear models test, as appropriate, revealed only a few statistically significant differences. These were for titers against *P. gingivalis* between baseline and 30 months ($p=0.0003$), for titers to *P. intermedia* between 4 and 30 months ($p=0.0261$), and for titers to *A. actinomycetemcomitans* ($p=0.0182$) and *A. naeslundii* ($p=0.042$) between baseline and 4 months. No apparent directional changes were observed over time in controls (right panels), and no statistically significant changes in titer levels between baseline and 30 months were identified.

Figure 2 illustrates the observed changes in antibody levels from baseline to 30 months in periodontitis patients with respect to the type of periodontal therapy rendered, i.e. in the 22 subjects who were treated exclusively by means of non-surgical periodontal therapy, the 18 subjects who were treated by scaling followed by periodontal surgery, and the 49 patients who received periodontal surgery and adjunctive systemic antibiotics. Data are presented in a logarithmic scale, negative values signify reductions in titer level from baseline to 30 months while positive values represent titer increases. No discernible pattern of titer change appeared to emerge for any of the three treatment modalities. In fact, analysis of variance of the observed changes in antibody titer between baseline and 30 months revealed no statistically significant differences among the three treatment modalities.

Table 2. Clinical characteristics in periodontitis patients and controls

Variable	Controls ($N=42$)	Patients ($N=89$)		
	baseline	baseline	4 months	30 months
no. of teeth	27.2 (1.3)	23.5 (4.4)	22.8 (4.7)	22.4 (5.0)
plaque (%)	36.4 (24.7)	47.6 (24.0)	36.9 (18.1)	21.7 (15.7)
bleeding on probing (%)	23.3 (12.3)	62.2 (21.1)	23.1 (14.2)	30.2 (16.5)
PD (mm)	2.0 (0.21)	3.6 (0.8)	2.9 (0.6)	2.5 (0.3)
no. of sites with PD$\geqslant$6 mm	0.0 (0.15)	21.5 (23.0)	7.0 (7.7)	1.2 (2.2)
AL (mm)	1.0 (0.6)	3.1 (1.2)	3.0 (1.1)	2.9 (1.0)
no. of sites with AL$\geqslant$6 mm	0.9 (4.3)	20.5 (19.0)	16.9 (16.8)	14.4 (16.1)

Mean values (standard deviations).PD, probing depth; AL, attachment level.

Discussion

Our data demonstrate an overall stability of serum antibody titers to periodontal bacteria over a 30-month period for both periodontitis patients and periodontally intact control subjects. Periodontal therapy generally had only a modest effect on antibody titers, and the induced changes in antibody levels from baseline to 30 months were not found to be different between patients who received non-surgical periodontal therapy, periodontal surgery, or surgery and adjunctive systemic antibiotics. Importantly, even after successful periodontal therapy, periodontitis patients were significantly different from the periodontally intact controls with respect to titers to several periodontal bacteria, notably to *A. actinomycetemcomitans* and ''red complex'' species. The latter observation underscores the value of serology in discriminating between subjects with periodontitis and individuals with low susceptibility to destructive disease.

The notion that periodontitis patients display higher serum IgG responses to specific bacteria than healthy individuals has wide support in the literature (Naito et al. 1984, Kinane et al. 1999, Craig et al. 2002). Furthermore, studies have documented differences between diseased and healthy subjects on the IgG subclass level (Lu et al. 1994, Pietrzak et al. 1998). Evidence of specific titers being elevated in periodontally healthy subjects is sparse, although the observed trend of higher titers against *P. melaninogenica* in control subjects has been earlier documented (Doty et al. 1982).

The literature is less unanimous on the issue of longitudinal stability of such titers after periodontal therapy. A majority of studies indicates that titers to important periodontal pathogens are substantially reduced after successful periodontal therapy (Aukhil et al. 1988, Johnson et al. 1993). Some studies point out that the effect of therapy on antibody levels may differ in subjects

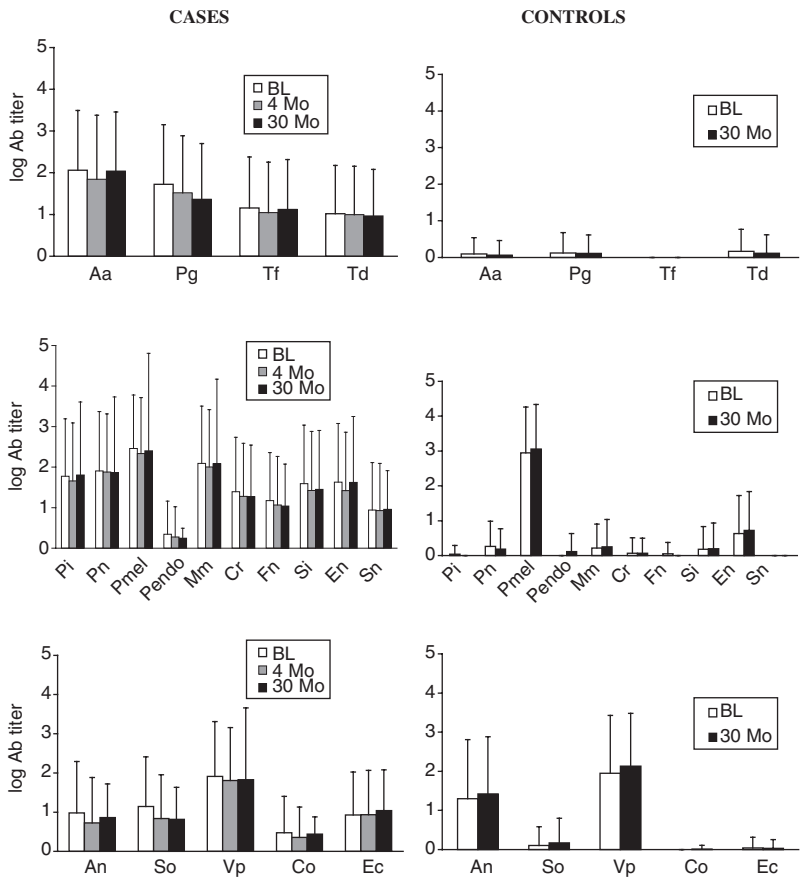

Fig. 1. Antibody responses (nanograms per milliliter expressed in a logarithmic scale) in periodontitis patients (''Cases'', left panel) and periodontally intact controls (right panel) at baseline, 4 months and 30 months. Abbreviations are as follows: Aa, *Actinobacillus actinomycetemcomitans*; Pg, *Porphyromonas gingivalis*; Tf, *Tannerella forsythensis*; Td, *Treponema denticola*; Pi, *Prevotella intermedia*; Pn, *Prevotella nigrescens*; Pmel, *Prevotella melaninogenica*; Pendo, *Porphyromonas endodontalis*; Mm, *Micromonas micros*; Cr, *Campylobacter rectus*; Fn, *Fusobacterium nucleatum*; Si, *Streptococcus intermedius*; En, *Eubacterium nodatum*; Sn, *Selenomonas noxia*; An, *Actinomyces naeslundii*; Si, *Streptococcus intermedius*; Vp, *Veillonella parvula*; Co, *Capnocytophaga ochracea*; Ec, *Eikenella corrodens*.

who are seropositive or seronegative prior to therapy, and that treatment induces an antibody level reduction in the former group of patients but an increase in the latter (Chen et al. 1991, Mooney et al. 1995). It should be noted, however, that such dichotomizations are rather arbitrary and largely inconsistent between studies, because they employ either internal thresholds values (i.e. a given percentile) or comparisons with pooled serum levels. Finally, an absence of a treatment effect on titers has also been reported (Sandholm & Tolo 1986).

In this study, we observed statistically significant reductions in only a few of the investigated titers after periodontal therapy, and no difference was observed in antibody changes over time between patients that received scaling alone, scaling and periodontal surgery, or scaling, surgery and adjunctive antibiotics. To properly interpret these results, however, a couple of points need to be emphasized: first, it must be stressed that the present study is not a randomized controlled trial of periodontal therapies. Patients were not randomly assigned to different modes of periodontal therapy, but received the therapy that their care-taking periodontist regarded as most suitable to their particular needs. This obviously entails that the severity/clinical presentation of the disease was a factor both in the decision to treat surgically or not, and to the decision to prescribe adjunctive antibiotics. With respect to the latter, it is clear that the study claims no aspirations to elucidate the impact of specific antibiotic regimens on serological markers of periodontitis, because their prescription neither randomized nor standardized.

It is also useful to evaluate the present serological data in conjunction with the microbiological data that are available from this subject cohort but are, for brevity, not included in this report. The subgingival bacterial profiles of the larger baseline case–control cohort were reported previously (Papapanou et al. 2000) and, rather expectedly, revealed that periodontitis patients harbored a significantly higher load by certain species including *P. gingivalis*, *T. forsythensis*, *T. denticola*, and *C. rectus*. In contrast, control subjects harbored significantly higher levels of *E. corrodens*, *V. parvula* and *A. naeslundii*. Clearly, the vast majority of control subjects were not free from the investigated species but were colonized at low levels. We re-examined the subgingival microbial profiles of the periodontitis patients after treatment at 4 and 30 months, and analyzed by checkerboard DNA–DNA hybridization (Socransky et al. 1994) a total of 14 individual subgingival plaque samples/subject with respect to the same 19 periodontal species (data not shown). In all three treatment groups, we observed that the median bacterial load per patient was substantially reduced between baseline and 4 months for all species. This reduction was most evident in the group that received adjunctive antibiotics. Nevertheless, all investigated bacterial species were still detectable at 4 months irrespective of treatment modality, and no species elimination/suppression below the detection level was achieved. Interestingly, despite the continuing improvement in probing depths and attachment levels between 4 and 30 months (Table 2), bacterial loads rebounded during the same period, paralleling the observed increase in plaque and gingivitis levels. As a result, no statistically significant reduction in bacterial load from baseline to 30 months was noted in any treatment group. Thus, the microbiological data provide an explanation for the persisting antibody titers in the treated subjects, suggesting that they remained continuously challenged by periodontal microbiota over the observation period, despite successful periodontal therapy.

Taken together, and in accordance to earlier reports (Vincent et al. 1987, Taubman et al. 1992), our findings suggest that periodontitis patients and subjects non-susceptible to periodontitis

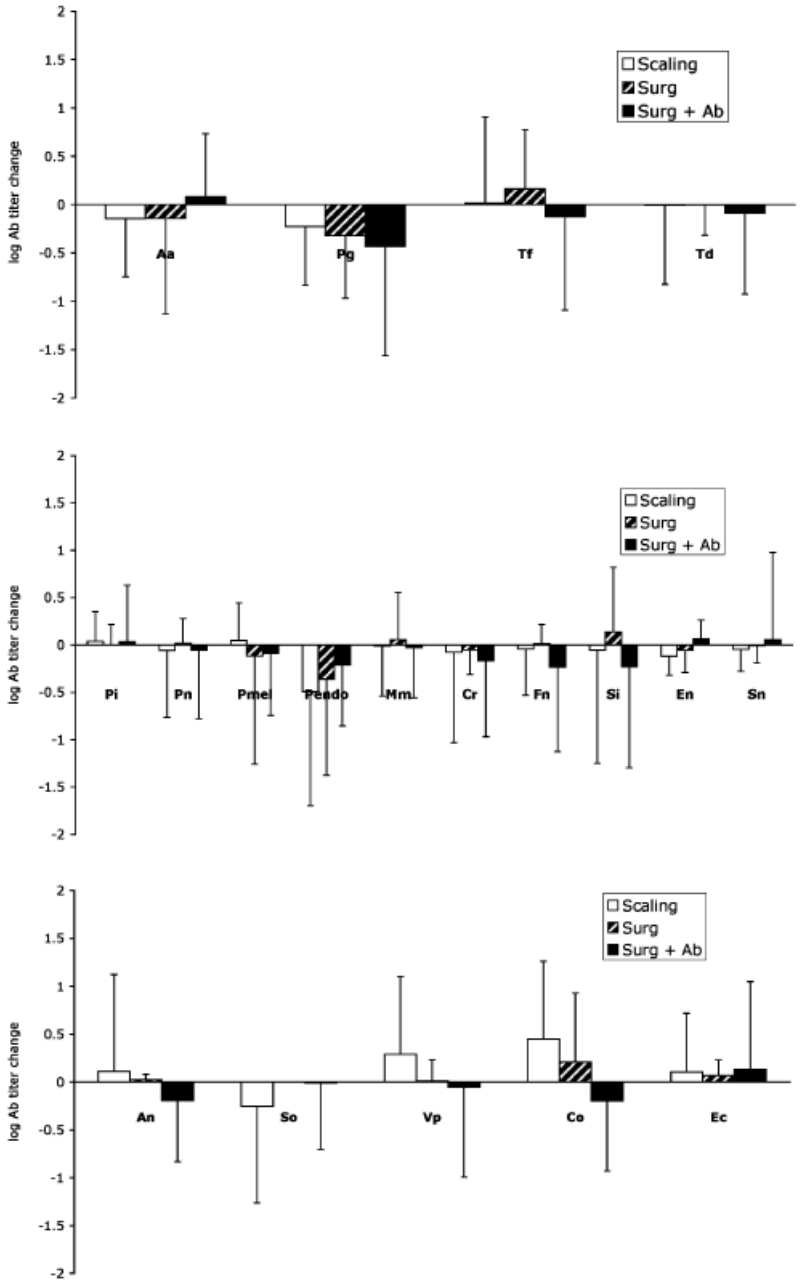

Fig. 2. Changes in antibody responses (nanograms per milliliter expressed in a logarithmic scale) in periodontitis patients between baseline and 30 months with respect to periodontal therapy rendered (scaling alone, scaling and periodontal surgery, or scaling, periodontal surgery and adjunctive systemic antibiotics). Positive values indicate an increase of antibody levels from baseline while negative values represent a reduction in antibody levels. Bacterial abbreviations as in Fig. 1.

elicit serum IgG responses to periodontal microbiota of clearly distinct intensities, and that these differences do persist after therapy. The observed discriminative potential of serum antibody responses and their relative stability over time suggest that serology to periodontal bacteria is a useful complement in the armamentarium of diagnostic tools for periodontal diseases.

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