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Quadrant root planing versus same-day full-mouth root planing

III. Dynamics of the immune response

D. A. Apatzidou[1] and D. F. Kinane[2]

[1]Periodontal and Oral Immunology Research Group, Glasgow Dental School, Glasgow, UK and [2]Periodontics, Endodontics and Dental Hygiene, University of Louisville School of Dentistry, Lovisville, KY, USA

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Abstract

Objectives: The aim of this study was to determine whether same-day full-mouth scaling and root planing (FM-SRP) and quadrant scaling and root planing (Q-SRP) resulted in variations in the systemic humoral immune response dynamics (antibody titres and avidity) during active treatment and 3 and 6 months post-therapy.

Material and Methods: Forty patients with chronic periodontitis were recruited into this study. Subjects were randomised into two groups and received either scaling and root planing quadrant by quadrant at 2-weekly intervals (Q-SRP group) or same-day full-mouth scaling and root planing (FM-SRP group). Clinical measurements and serum samples were obtained at baseline and approximately 6 weeks after the last clinical intervention (R1) and 6 months after the initiation of therapy (R2). Furthermore, serum samples were obtained from each patient undergoing therapy (Q-SRP and FM-SRP) at 3 bi-weekly instances so as to determine the short-term effects of each session of scaling and root planing on the dynamics of the humoral immune response. Serum antibody titre was assayed by enzyme-linked immunosorbent assay (ELISA) and antibody avidity was measured by thiocyanate dissociation against five putative periodontal pathogens: *Porphyromonas gingivalis*; *Actinobacillus actinomycetemcomitans*; *Prevotella intermedia*; *Treponema denticola* and *Bacteroides forsythus*.

Results: Both therapies resulted in similar antibody titre reductions against the majority of the organisms tested and although there was a distinct trend for antibody avidity to increase following therapy, this was not found to be statistically significant, reflecting marked inter-individual variation. In addition, no evidence emerged from this study to support increased antibody titres following the active phases of both treatment approaches due to an inoculation effect. Nevertheless, significant short-term increases in antibody avidity to most test bacteria were noted for both treatment strategies.

Conclusion: Both therapies were associated with a reduction in antibody titres and an increase in the binding ability or avidity of antibodies, but there was a marked inter-subject variability and statistical significance was reached for only some of the test bacteria. No significant differences in the humoral antibody dynamics were found between the two treatment approaches.

Key words: antibody avidity; antibody titres; chronic periodontitis; full-mouth periodontal therapy; periodontal pathogens; quadrant scaling and root planing

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Several microbial species are considered pathogens relevant to chronic periodontitis and these include *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Bacteroides forsythus*, *Prevotella intermedia*, and *Treponema denticola* (AAP 1996). Studies have shown that a significant antibody response is directed towards *P. gingivalis* and *A. actinomycetemcomitans* (Mouton et al. 1981, Ebersole et al. 1982, Kinane et al. 1993, Mooney & Kinane 1994). Antibody responses towards *P. intermedia*, *B. forsythus* and *T. denticola* have also been demonstrated in periodontal patients (Jacob et al. 1982, Ebersole et al. 1986, Zafiropoulos et al. 1992, Califano et al. 1997).

In general, the antibody response is considered to be protective (Mooney & Kinane 1997) and the effect of scaling

and root planing on changing the humoral immune response has produced mixed reports. Tolo et al. (1982) found both increases and decreases in antibody titres to different organisms, and Ebersole et al. (1985) showed a marked increase in antibody titres to *P. gingivalis*, *P. intermedia* and *A. actinomycetemcomitans* post-therapy. Similarly, Mooney et al. (1995) showed a significant increase in antibody titres to *A. actinomycetemcomitans* and *P. gingivalis*, following therapy. However, Aukhil et al. (1988), Murray et al. (1989), and Horibe et al. (1995) reported reductions in titres to *P. gingivalis* and *P. intermedia* after therapy.

Antibody avidity is a measure of the net binding strength between multivalent antigens and polyclonal antibodies. It reflects the functional activity of the antibodies and as a consequence, the maturation of the immune system. Chen et al. (1991) investigated the humoral immune response in generalised aggressive periodontitis patients and concluded that many patients do not produce protective levels of biologically functional antibody of high avidity during the course of their natural infection, but treatment may induce the production of such antibodies. Whitney et al. (1992) investigated the humoral immune response against a whole-cell homogenate of *P. gingivalis* in generalised aggressive periodontitis patients, as well as in control subjects. In general, low titre and low avidity anti-*P. gingivalis* IgG antibodies were observed in generalised aggressive periodontitis patients, indicating a genetically determined compromise of their humoral immune response to certain types of antigens.

A recent study from our laboratory showed that conventional periodontal treatment of chronic periodontitis patients resulted in significant clinical improvements and significant reductions in some of the test organisms (*P. intermedia*, *B. forsythus* and *T. denticola*) (Darby et al. 2001). There was little change, however, in the systemic and local antibody titres post-scaling, although there was a significant reduction in the IgG antibody avidity to *P. gingivalis* and *P. intermedia*. These results indicate complex interactions between the subgingival microflora and the host response and also a possible failure of the host response to produce adequate levels of biologically functional antibodies to putative periodontal pathogens.

Quirynen et al. (1995) performed a comparative study of full-mouth root planing and the standard quadrant root planing at 2-weekly intervals, in 10 periodontitis patients. This study reported benefits for the full-mouth approach in terms of pocket depth (PD) reductions and microbiological improvements. In their recent study, Quirynen et al. (2000) noted that seven out of 11 patients, whose body temperature rose above 37°C after the second day, had an overall average PD reduction of 3.5 mm, whereas this was only the case for four of the remaining 13 patients who did not develop the increase in temperature. The observation that patients with some rise in body temperature the evening after the second day of the full-mouth treatment were the subjects who showed more impressive improvements is considered by Quirynen to be due to an increased immunological response in these patients.

The aim of the present study was to examine the effect of quadrant scaling and root planing (Q-SRP) and full-mouth scaling and root planing (FM-SRP) on the dynamics of the humoral immune response to *A. actinomycetemcomitans*, *P. gingivalis*, *B. forsythus*, *P. intermedia* and *T. denticola* (both antibody titre and avidity) during and following the active phase of therapy. In addition, immunological variations between the two treatment approaches were compared with test the hypothesis that FM-SRP causes an acute immunological reaction that gives an enhanced clinical outcome.

Material and Methods

Forty untreated chronic periodontitis patients, aged 31–70 years, were recruited from new referrals to Glasgow Dental Hospital and School and attended for the 6-month duration of the study. Each patient had at least two non-adjacent sites per quadrant on different teeth with a PD of 5 mm or over and radiographic evidence of bone loss with no history of systemic disease or antibiotic therapy within the last 3 months or during the course of the study. All patients gave informed consent.

The demographic details and clinical study design are described in our previously published report (Apatzidou & Kinane 2003). The trial design and the timings of clinical interventions and assessments are summarised in Tables 1 and 2. FM-SRP or Q-SRP was performed on each patient by an experienced periodontist (D. A. A.). Briefly, the treatment protocol was as follows: Q-SRP was performed quadrant by quadrant over four consecutive sessions at 2-weekly intervals, while FM-SRP was completed within the same day (i.e. 12 h). No use of disinfection i.e. antiseptics such as chlorhexidine was used in either treatment group. No teeth were extracted during therapy in order to avoid bias. Subsequent to the same-day FM-SRP, patients in this treatment group were recalled at 2-weekly intervals for oral hygiene instructions (OHI). Oral hygiene reinforcement was carried out at each visit for all participants. Effectively, patients in both treatment groups were seen at equal time points and received an equal amount of OHI and motivation.

The subjects were assessed clinically by a calibrated single examiner (D. A. A.) at three time points: at baseline (BAS), at reassessment 1 (R1), approximately 6 weeks after the last clinical intervention and at reassessment 2 (R2), 6 months from baseline. Furthermore, at these time points, subgingival plaque samples were collected for the detection of five putative periodontal pathogens: *A. actinomycetemcomitans*, *P. gingivalis*, *B. forsythus*, *P. intermedia* and *T. denticola* by polymerase chain reaction (PCR) as described previously (Apatzidou et al. 2003). In addition, at each clinical session a blood sample was collected from all participants to determine the serum antibody titres and avidity against the homologous organisms.

Blood Sampling and Processing of Sera Samples

Venous blood was collected from the ante-cubital vein by venipuncture using the Vacutainer system (BD Vacutainer™, Plymouth, UK). Three tubes of 7 ml of venous blood were collected. Each blood sample was allowed to clot overnight in tubes with no additive and then centrifuged at 2000 rpm for 10 min. The serum was aliquoted at a volume of 1 ml and stored into labelled microcentrifuge tubes at −70°C for further analysis. The aliquots were then coded so that the laboratory analysis was performed in a blind manner.

Preparation of Microorganisms and Coating of Plates

A. actinomycetemcomitans, *P. gingivalis*, *P. intermedia*, *B. forsythus* and *T.*

Table 1. Schedule of assessments for the Q-SRP group

Q-SRP assessment	BAS	Treatment phase				Follow-up phase	
		T1	T2	T3	T4	R1	R2
week number	0w	1w	3w	5w	7w	13w	25w
visit number	− 1	1	2	3	4	5	6
inclusion/exclusion criteria	√						
informed consent/randomisation							
pocket chart (PD, CAL, BOP)	√					√	√
selected-site clinical recordings	√		√	√	√	√	√
plaque samples	√					√	√
blood sample	√		√	√	√	√	√
quadrant scaling/root planing (Q-SRP)		√	√	√	√		
oral hygiene instructions		√	√	√	√	√	
questionnaire		√					
maintenance scaling						√	

Q-SRP = quadrant scaling and root planing; baseline = BAS; PD = pocket depth; CAL = clinical attachment level; BOP = bleeding on probing; R1 = reassessment 1; R2 = reassessment 2.

Table 2. Schedule of assessments for the FM-SRP group

FM-SRP assessment	BAS	Treatment phase	Follow-up phase				
		T1	F1	F2	R1	F3	R2
week number	0w	1w	3w	5w	7w	13w	25w
visit number	− 1	1	2	3	4	5	6
inclusion/exclusion criteria	√						
informed consent/randomisation							
pocket chart (PD, CAL, BOP)	√				√		√
selected-site clinical recordings	√				√		√
plaque samples	√				√		√
Blood sample	√		√	√	√	√	√
full-mouth scaling/root planing (FM-SRP)		√					
oral hygiene instructions		√	√	√	√	√	
questionnaire		√					
maintenance scaling						√	

FM-SRP = full-mouth scaling and root planing; BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

denticola were prepared for coating the enzyme-linked immunosorbent assay (ELISA) plates. *A. actinomycetemcomitans* strain Y4 was grown on blood agar plates and harvested after 24 h. *P. gingivalis* strain W50, *P. intermedia* strain ATCC 25611 and *B. forsythus* strain ATCC 43037 were grown on fastidious anaerobe agar and harvested after 7 days. The organisms were harvested with swabs and dispersed into PBS containing 0.1 mM disodium EDTA (PBSE). They were then washed once in PBSE and fixed overnight in 10% formal saline. *T. denticola* strain ATCC 35405 cells were grown, fixed and kindly donated by Dr. C. Wyss (Zurich, Switzerland). After washing twice with PBSE, the fixed organisms were re-suspended in coating buffer (CB). The organisms were further washed once with CB, and were used to coat the plates at bacterial concentrations determined by the following optical densities (OD_{600}): 0.02 for *A. actinomycetemcomitans*; 0.05 for *P. gingivalis*; 0.05 for *P. intermedia*; 0.02 for *B. forsythus*; and 0.001 for *T. denticola*.

Enzyme-Linked Immunosorbent Assay (ELISA)

Specific antibody titres were measured by ELISA as previously described (Ebersole et al. 1980), using formalised whole cells at an absorbance that was previously determined as optimal to coat microtitre plates. Immulon 1 plates (Dynatech, Billingshurst, UK) were used because of their low protein-binding characteristics. The plates were manually pre-washed three times with CB and coated with 100 μl per well of whole cells. Plates were stored at 4°C overnight. Control wells for each aspect of the ELISA process were arranged in the wells around the outside of the plates.

After coating, the plates were washed five times with 250 μl of wash buffer using an automatic washer (Dynex Ultrawash Plus, Ashford, England). Then they were treated with 100 μl per well of incubation buffer (IB) containing 5% skimmed milk (Marvel, Premier Beverages, Stafford, UK) for 30 min at 37°C to remove background binding. The plates were washed again five times before the addition of sera. Serum serially diluted from 1/100 to 1/25,600 in IB was used as a reference positive control serum. Serum derived from the study patients was added at a dilution of 1/200. Fifty microlitres of sera were added in each well and was incubated at 37°C for 90 min. Following incubation with sera, the plates were washed again five times. Subsequently, 100 μl per well of biotin-conjugated anti-human IgG (Sigma, St Louis, MI, USA) at 1/2000 dilution in IB was added and

plates were incubated at 37°C for 60 min. The plates were then washed five times. Thereafter, they were incubated at 37°C for 60 min or at 4°C overnight with 100 μl per well extravidin-peroxidase (Sigma) at 1/2000 dilution. After washing (five times), the reaction was visualised using 100 μl per well tetramethylbenzidine (TMB) (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA) and stopped after 5–10 min using 50 μl 0.1 M HCl.

ODs were read using a Dynex Technologies MRX II plate reader at 450 nm with a 630 nm reference wavelength. All sera samples collected from the same patient at different time points (six samples in total) were assayed in duplicate and on the same plate. Correction was made for non-specific binding and the averaged duplicate results were read from a reference line derived from serial dilutions of the reference positive control serum. The results were expressed as ELISA units (EU) (Gmür et al. 1986, Mooney et al. 1993).

Avidity Analysis

The dissociation assay to determine antibody avidity was performed in a similar manner to the ELISA for the serum analysis described above. After incubation with sera, the wells were treated with increasing concentrations of ammonium thiocyanate (0, 0.2, 1.0, 2.0, 3.0 M). Patient serum was tested in duplicate at a dilution of 1/20, and the thiocyanate dilutions were compared with a buffer blank with 100% binding. The plates were incubated at 37°C for 60 min, washed five times automatically and then the ELISA continued as before. The concentration of thiocyanate as a molarity (M) required to dissociate 50% of the bound antibody was calculated by linear regression. This concentration was termed the 50% inhibitory dose (ID_{50}) and provided a measure of the relative avidity (Pullen et al. 1986, MacDonald et al. 1988). All the samples from the same patient were tested together on the same plate.

Statistical Analysis of Data

The data were analysed using Minitab statistical package (Minitab release 12, State College, PA, USA). Statistical significance was set at the 95% confidence level ($p < 0.05$ for hypothesis testing).

The Mann–Whitney test was used for the comparison of serum IgG titres, and IgG avidity between the two groups at baseline and after treatment. This test analysed the data for the detection of differences between the two groups at each visit (BAS, R1, R2) and for comparison of changes with treatment (BAS to R1, BAS to R2). Within each group, the Wilcoxon signed-rank test was used to assess changes before and after treatment.

For the determination and comparison of the humoral immune response dynamics between the Q-SRP group and FM-SRP group, the Mann–Whitney test analysed and compared the changes in IgG antibody titres and avidities at each visit from baseline (baseline against five additional visits). The Wilcoxon signed-rank test was used to determine the immune response dynamics in each treatment group by comparing baseline with each visit.

Results

In general, there was a decrease in serum IgG antibody titres against the five bacteria (expressed as EU) following Q-SRP (Table 3). For *P. gingivalis*, there was a statistically significant decrease in the antibody levels at R2 from baseline ($p < 0.005$). For *P. intermedia* and *T. denticola*, a reduction in serum antibody titres was noted at R2, but these findings just failed to reach statistical significance ($p = 0.050$ for *P. intermedia*, $p = 0.052$ for *T. denticola*). No significant reductions in antibody levels were noted for *A. actinomycetemcomitans* and *B. forsythus* following treatment. Despite the general trend for

Table 3. Comparison of serum IgG antibody titres before and after Q-SRP and FM-SRP

$N_{Q\text{-}SRP} = 20$ $N_{FM\text{-}SRP} = 20$	BAS	R1	R2	Change (BAS–R1)	Change (BAS–R2)
Porphyromonas gingivalis					
Q-SRP	149.0 (42.0, 809.0)	81.0 (29.0, 843.0)	72.0 (21.0, 535.0)	11.0 (−20.0, 93.0)	49.0 (2.0, 387.0)§§
FM-SRP	80.0 (32.0, 500.0)	88.0 (33.0, 794.0)	137.0 (28.0, 275.0)	5.0 (−33.0, 125.0)	16 .0 (−38.0, 739.0)
Actinobacillus actinomycetemcomitans					
Q-SRP	63.0 (18.0, 211.0)	52.0 (20.0, 394.0)	35.0 (21.0, 175.0)	4.0 (−16.0, 54.0)	8.0 (−9.0, 36.0)
FM-SRP	64.0 (12.0, 1312.0)	41.0 (15.0, 554.0)	57.0 (15.0, 945.0)	2.0 (−2.0, 201.0)	9.0 (−3.0, 786.0)§
Prevotella intermedia					
Q-SRP	144.0 (64.0, 403.0)	131.0 (50.0, 694.0)	101.0 (47.0, 399.0)	6.0 (−67.0, 42.0)*	30.0 (−13.0, 131.0)
FM-SRP	298.0 (86.0, 735.0)	244.5 (81.7, 404.0)	328.0 (121.0, 467.5)	53.0 (−6.0, 237.0)§§,*	14.0 (−47.0, 206.0)
Treponema denticola					
Q-SRP	13.0 (5.0, 75.0)	15.0 (3.5, 58.5)	12.5 (1.5, 56.5)	0.5 (−19.0, 3.0)*	2.5 (0.0, 12.8)
FM-SRP	18.0 (8.0, 60.0)	10.5 (6.0, 28.0)	14.5 (7.5, 26.0)	6.5 (0.3, 36.0)§§,*	5.5 (0.8, 48.0)§
Bacteroides forsythus					
Q-SRP	33.0 (17.0, 1156.0)	29.0 (14.0, 1007.0)	37.0 (13.0, 542.0)	−1.0 (−7.0, 71.0)	6.0 (−1.0, 60.0)
FM-SRP	32.0 (14.0, 676.0)	33.0 (12.0, 265.0)	32.5 (14.5, 194.5)	7.0 (−5.0, 84.0)	2.0 (−5.0, 183.0)

Serum IgG titres are expressed as ELISA units (EU).

Median (interquartile range)

*$p < 0.05$; *p*-values represent differences between Q-SRP and FM-SRP groups.

§$p < 0.05$;

§§$p < 0.01$; *p*-values represent longitudinal changes from baseline within each treatment group.

Q-SRP = quadrant scaling and root planing; FM-SRP = full-mouth scaling and root planing; BAS = baseline; R1 = reassessment 1; R2 = reassessment 2; ELISA = enzyme-linked immunosorbent assay.

Table 4. Comparison of IgG antibody avidity before and after Q-SRP and FM-SRP

$N_{Q\text{-}SRP} = 20$ $N_{FM\text{-}SRP} = 20$	BAS	R1	R2	Change (BAS–R1)	Change (BAS–R2)
Porphyromonas gingivalis					
Q-SRP	0.46 (0.28, 0.63)	0.45 (0.31, 0.64)	0.49 (0.31, 0.91)	− 0.02 (− 0.13, 0.08)	− 0.06 (− 0.26, 0.06)
FM-SRP	0.49 (0.34, 0.61)	0.51 (0.33, 0.67)	0.56 (0.31, 0.72)	0.03 (− 0.13, 0.13)	− 0.02 (− 0.07, 0.15)
Actinobacillus actinomycetemcomitans					
Q-SRP	0.38 (0.33, 0.55)	0.40 (0.28, 0.53)	0.42 (0.30, 0.70)	0.02 (− 0.06, 0.10)	0.44 (0.38, 0.57)
FM-SRP	0.44 (0.38, 0.57)	0.44 (0.40, 0.84)	0.57 (0.41, 0.88)	− 0.04 (− 0.21, 0.05)	− 0.13 (− 0.33, 0.03)*
Prevotella intermedia					
Q-SRP	0.84 (0.59, 1.00)	0.72 (0.58, 0.88)	0.86 (0.68, 1.12)	0.08 (− 0.09, 0.31)	0.01 (− 0.19, 0.08)
FM-SRP	0.84 (0.65, 1.00)	0.88 (0.68, 1.30)	0.83 (0.67, 1.20)	− 0.01 (− 0.10, 0.08)	0.06 (− 0.10, 0.11)
Treponema denticola					
Q-SRP	0.30 (0.22, 0.36)	0.31 (0.25, 0.54)	0.33 (0.25, 0.43)	− 0.04 (− 0.23, 0.02)	− 0.03 (− 0.08, 0.04)
FM-SRP	0.36 (0.26, 0.53)	0.45 (0.35, 0.74)	0.40 (0.31, 0.46)	− 0.10 (− 0.26, 0.07)	− 0.01 (− 0.12, 0.05)
Bacteroides forsythus					
Q-SRP	0.37 (0.22, 0.65)	0.41 (0.26, 0.56)	0.38 (0.27, 0.69)	0.00 (− 0.15, 0.12)	0.00 (− 0.09, 0.05)
FM-SRP	0.46 (0.35, 0.58)	0.46 (0.29, 0.60)	0.46 (0.33, 0.76)	0.02 (− 0.08, 0.11)	− 0.03 (− 0.20, 0.05)

IgG avidity is expressed as molarity (M) of ammonium thiocyanate at ID_{50}.
Median (interquartile range).
No statistically significant differences were noted between Q-SRP and FM-SRP groups at any time point.
*$p < 0.05$; *p*-values represent longitudinal changes from baseline within each treatment group.
Q-SRP = quadrant scaling and root planing; FM-SRP = full-mouth scaling and root planing; BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

IgG avidity (expressed as M at ID_{50}) to increase 6 months post-treatment, changes did not reach statistical significance (Table 4).

In a similar way to Q-SRP, same-day FM-SRP resulted in significantly lower serum IgG antibody titres (EU) after treatment for the majority of the bacteria tested (Table 3). Nevertheless, no significant decreases were noted for *P. gingivalis* and *B. forsythus* at R1 and R2. The median IgG antibody titres to *A. actinomycetemcomitans* significantly decreased at R2 ($p < 0.05$) and antibody levels to *P. intermedia* significantly decreased at R1 from baseline ($p < 0.01$). The median IgG titres against *T. denticola* were significantly reduced at R1 ($p < 0.01$) and R2 ($p < 0.05$). IgG avidity (M at ID_{50}) tended to increase post-treatment for the majority of the organisms, but this observation was statistically significant for antibodies against *A. actinomycetemcomitans* only ($p < 0.05$) (Table 4).

Tables 3 and 4 show that there were no statistically significant differences in serum IgG antibody titres (EU) and avidity (M at ID_{50}) between the two treatments at baseline and at R1 and R2. However, when the changes at R1 and R2 from baseline were examined and compared between the treatment groups, there was a significantly greater reduction in serum IgG antibody titres against *P. intermedia* and *T. denticola* at R1 for the FM-SRP group ($p < 0.05$) (Table 3). No significant differences in the changes of IgG avidity with treatment were seen between Q-SRP and FM-SRP groups (Table 4).

Humoral Immune Response Dynamics after Q-SRP and FM-SRP

In general, serum IgG antibody titres to all pathogens tested did not appear to increase significantly during the active phase of treatment (Q-SRP and FM-SRP) with reference to baseline. For the purpose of illustrating the dynamics of the humoral response across the treatment groups, the response to *T. denticola* is shown as this was the typical response. The responses to the other pathogens at BAS, R1 and R2 can be viewed in Tables 3 and 4. Figs. 1 and 2 illustrate the dynamics of antibody titres to *T. denticola* over a 6-month period for the two treatment groups. The analysis of the IgG antibody avidity (M at ID_{50}) over the six visits for the Q-SRP group showed significant increases to the majority of the test organisms during the active phase of treatment and the changes in antibody avidity to *T. denticola* are illustrated in Figs. 3 and 4.

Discussion

In general, decreases in serum IgG antibody titres to all putative pathogens were seen at 6 months with only some of them reaching statistical significance. These post-treatment reductions paralleled decreases in the detection frequency

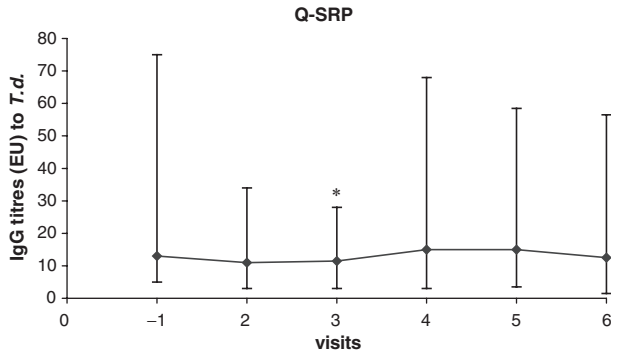

Fig. 1. IgG titres (ELISA units, EU) to *Treponema denticola* (*T.d.*) during the six visits for the quadrant scaling and root planing (Q-SRP) group. Median (interquartile range). *$p < 0.05$; *p*-values represent changes from visit − 1 (baseline).

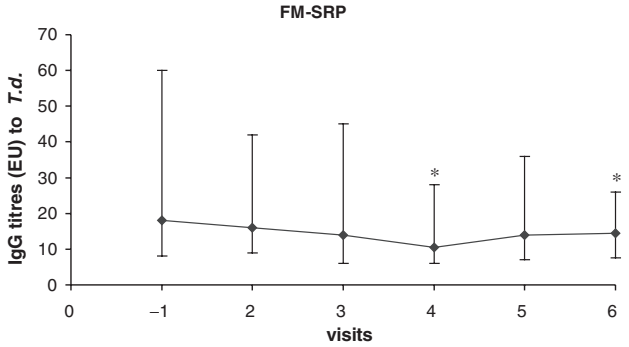

Fig. 2. IgG titres (ELISA units, EU) to *Treponema denticola* during the six visits for the full-mouth scaling and root planing (FM-SRP) group. Median (interquartile range). $^{*}p<0.05$; *p*-values represent changes from visit −1 (baseline).

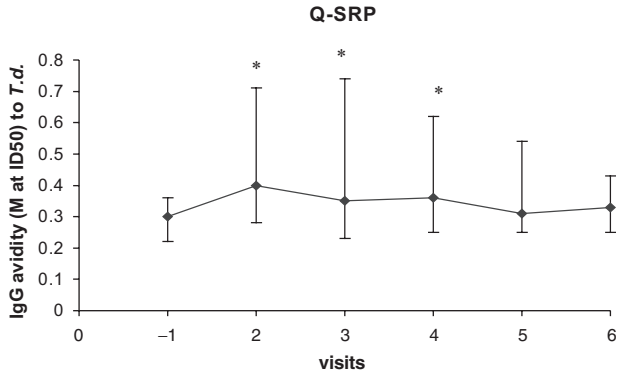

Fig. 3. IgG avidity (M at ID_{50}) to *Treponema denticola* during the six visits for the quadrant scaling and root planing (Q-SRP) group. Median (interquartile range). $^{*}p<0.05$; *p*-values represent changes from visit −1 (baseline).

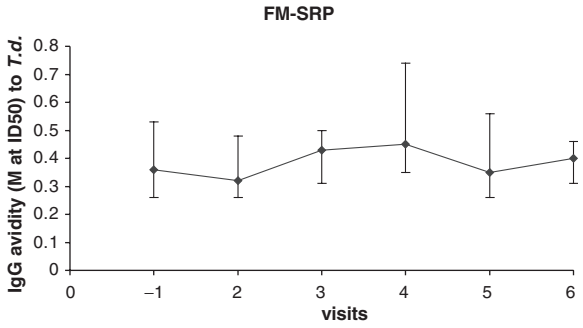

Fig. 4. IgG avidity (M at ID_{50}) to *Treponema denticola* during the six visits for the full-mouth scaling and root planing (FM-SRP) group. Median (interquartile range). *No statistically significant changes were noted at each visit from visit −1 (baseline).

of the homologous species in subgingival plaque (Apatzidou et al. 2003). The current results agree with the data reported by other investigators (Tolo et al. 1982, Naito et al. 1985, Mouton et al. 1987, Aukhil et al. 1988, Murray et al. 1989, Horibe et al. 1995). However, a significant variation exists between studies with respect to the duration of the treatment and the sampling intervals. In the current study, scaling and root planing were completed within 6 weeks for the Q-SRP group and within a day for the FM-SRP group. Both treatments resulted in decreased serum antibody titres, with the more marked reductions seen at 6 months. Most of the studies mentioned showed reductions in the antibody titres over a period of 12 months, but it has to be taken into account that the active phase of treatment in these studies lasted notably longer than in the present study. However, other studies have demonstrated decreases in serum antibody levels in the immediate post-treatment period (Aukhil et al. 1988, Horibe et al. 1995).

Nevertheless, the present findings are inconsistent with those of Ebersole et al. (1985), who showed that antibody titres increased 2–4 months post-scaling and returned to pre-scaling levels 8–12 months after treatment. In the current study, no significant increases in IgG titres to any of the organisms tested were detected post-scaling, and this does not support the inoculation effect of root planing on the host tissues as suggested by Ebersole et al. (1985). However, since the participants in this study were followed over a period of approximately 6 months, no long-term comparisons can be made between the present findings and those of Ebersole et al. (1985). Similarly, increases in serum IgG antibody titres to *A. actinomycetemcomitans* with decreases in PDs post-treatment have been demonstrated (Sjöström et al. 1994). The main difference between that study and the current study is that the former investigation examined a group with generalised aggressive periodontitis patients who required extensive and repeated treatment over a period of time, and this may have boosted their humoral immune response. Mooney et al. (1995) showed elevated antibody titres to *A. actinomycetemcomitans* 6 weeks after the completion of therapy. Their results, in addition to those of Chen et al. (1991), showed increased antibody titres to *A. actinomycetemcomitans* and *P. gingivalis*, respectively, for the seronegative patients after treatment.

Successful treatment results in the elimination of the aetiological agents and maturation of the immune system to produce antibodies of high avidity (Chen et al. 1991). Mooney et al. (1995) found an increase in IgG avidity to *P. gingivalis* ($p = 0.05$) 6 weeks after hygiene phase therapy. In the present study, IgG avidity tended to increase post-treatment for the majority of the organisms tested, but generally this finding did not reach statistical significance for most of the test bacteria. It has been confirmed by other studies that antibody avidity is independent of the antibody levels (O'Dell & Ebersole,

1995). Data reported here show that treatment resulted in lower levels of antibodies to all the bacteria tested, but avidity remained the same or increased for the majority of the organisms tested.

Comparison of Immunological Parameters in Q-SRP and FM-SRP Groups

The current study showed no significant differences in IgG avidity between the two treatments. Although antibody titres were similar for both groups at baseline, R1 and R2, a significantly greater reduction in the antibody levels to *P. intermedia* and *T. denticola* was seen between baseline and R1 for the FM-SRP group than the Q-SRP group. When the changes in the serum antibody response with treatment were considered within each group, significant reductions in the antibody levels to *P. intermedia* and *T. denticola* were seen between baseline and R1 for the FM-SRP group, but this was not found for the Q-SRP group. These findings imply that FM-SRP seems to have a stronger short-term effect on the systemic antibody response compared with the classical therapy of quadrant root planing at 2-weekly intervals. However, the clinical significance of this finding is difficult to assess.

It was of interest to note that FM-SRP resulted in significantly lower percentages of patients positive for *P. intermedia* at R1 and a greater reduction in the detection frequency of *T. denticola* at R1 and R2 from baseline than did Q-SRP, as shown in our previous study (Apatzidou et al. 2003). These changes paralleled the significantly greater reductions in serum antibody levels to these pathogens at this time interval for the FM-SRP group compared with the Q-SRP group. This finding is in agreement with previous reports showing that reductions in the serum antibody levels post-treatment reflect reductions in the antigenic load (Mouton et al. 1987, Aukhil et al. 1988, Murray et al. 1989).

Dynamics of Antibody Response after Q-SRP and FM-SRP

There is evidence of a short-term effect of treatment on the antibody response. Horibe et al. (1995) found a significant reduction in the antibody levels at least 2 months after the completion of treatment. Aukhil et al. (1988) showed significantly lower antibody levels by the end of the hygiene phase of treatment, i.e. approximately 2 months after baseline. The current study is the first that has attempted to determine the dynamics of the antibody response during the active phase of treatment, i.e. at 2-weekly intervals over a period of 3 months. The data reported here failed to show that each session of root planing resulted in increased antibody levels due to an inoculation effect of bacteria in the host tissues as suggested by Ebersole et al. (1985). Each session of root planing seemed to result in antibodies of either similar or significantly lower levels compared with baseline values. These results agree with those of Mouton et al. (1987), who demonstrated a gradual reduction in antibody levels 5–7 months after treatment with no peak antibody levels seen in the immediate post-scaling period.

Antibody avidity appeared to increase during the active phase of treatment for both treatment groups and this reached statistical significance even after the first scaling visits, reflecting the production of high avidity antibodies as a result of antigenic elimination and/or immune maturation. However, of great interest was the observation that antibody avidity significantly increased in the immediate post-scaling period rather than at later stages (R1 and R2). This finding, if upheld by further studies, would contradict the hypothesis that the baseline values of antibody avidity in periodontitis patients exceed a threshold beyond which no significant increases can be noted as a result of a chronic disease process. However, it is difficult to interpret these results given the variations within and between individuals and over time.

No significant differences in serum antibody levels and avidity were seen between the two treatment groups at each visit during the active phase of treatment (baseline and three additional visits). However, comparison of changes in serum antibody levels and avidity with treatment between Q-SRP and FM-SRP groups revealed a significantly greater reduction in serum antibody levels to *T. denticola* for the FM-SRP group between baseline and visit 4, and a significantly lower increase in antibody avidity to *T. denticola* for the FM-SRP group between BAS and visit 2. It must be stressed, however, that visit 4 corresponded to R1 for the FM-SRP group and to the last session of quadrant root planing for the Q-SRP group. This means that the assessment of the systemic immunological parameters at this time point was made before treatment was completed in the Q-SRP group. These differences in the changes of antibody levels and avidity between the two treatment groups would appear to be of doubtful clinical significance.

It would be interesting to pursue a research protocol with more frequent blood sampling to examine the dynamics of the humoral immune response in greater detail. Our experience is that short-term perturbations of antibody responses occur, but show great variation across subjects and are thus difficult to interpret meaningfully. In this study, we were looking for longer-term changes, which could be linked to longer-term clinical changes. The current study did, however, use frequent blood samples (2-weekly intervals) over a long period (overall 6 months) than has been previously performed. A future investigation may utilise a more extensive series of frequent short-interval samples, which could also be useful for bacteraemia assessment.

Conclusion

The present findings did not confirm the hypothesis that the beneficial effects of FM-SRP with and without the use of chlorhexidine are due to an acute immunological reaction caused by the inoculation of bacteria into the host tissues (Quirynen et al. 2000). Furthermore, our data failed to show that same-day FM-SRP induced an increased antibody response that differs from that elicited by Q-SRP at 2-weekly intervals. The Leuven research group used more severely affected periodontitis patients than in the current study, thus having a greater potential for bacteraemia post-root planing. However, they recorded clinical indices from the maxillary right quadrant only and this makes comparisons with the present study difficult.

In conclusion, periodontal therapy undoubtedly decreases the antigenic load post-treatment and as a consequence the antibody levels to specific organisms. However, a wide variation in the subjects' antibody response to treatment was noted and can be partially explained by the large age range (31–70 years). A larger sample size, shorter intervals than 2 weeks for blood sampling and a longer examination period would be suggested for further investigation.

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Address:
Denis F. Kinane
Department of Periodontics,
Endodontics and Dental Hygiene
School of Dentistry
501 S. Preston
Louisville, KY 40202
USA
email: denis.kinane@louisville.edu