

# Plasma antibody levels in periodontitis patients and controls

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## Abstract

**Background:** A major aspect of the adaptive host response in periodontitis is the production of antibodies. Several risk and susceptibility factors for periodontitis, including smoking, age and composition of the subgingival microflora, have also been suggested to influence antibody production.

**Aim:** The present study was conducted to investigate plasma levels of immunoglobulin (Ig) G, A and M antibodies in periodontitis patients of Caucasian European heritage in relation to disease severity, smoking, diagnosis and prevalence of periodontopathogens.

**Methods:** In this study, 29 patients with severe periodontitis, 51 with moderate periodontitis and 55 controls without periodontal destruction were enrolled. From the total of 80 patients, 18 were diagnosed with aggressive periodontitis and 62 with chronic periodontitis. Total IgG, IgA and IgM as well as IgG isotypes were analyzed in plasma samples.

**Results:** Levels of total IgG, IgA and IgM were not different between patients and controls; however, in periodontitis, higher levels of IgG1 and IgG2 were observed. Smoking appeared to be significantly and inversely related to antibody levels in periodontitis, in particular for total IgG and IgG2. The absence of an elevated total IgG and IgG2 in smoking patients was irrespective of severity, prevalence of periodontal pathogens and diagnosis. The elevation of total IgG and IgG1 and IgG2 in non-smoker periodontitis patients was observed in patients with moderate periodontitis and even greater in patients with severe periodontitis, but was independent whether patients were infected with *Actinobacillus actinomycetemcomitans* or *Porphyromonas gingivalis* and independent of diagnosis. Clinically, it was observed that patients who smoked had more periodontal bone loss; the current findings on antibody levels may be one of several mechanisms related to more extensive periodontal breakdown in smoker patients.

**Conclusion:** The current study shows that non-smoker periodontitis patients have higher levels of total IgG and IgG2 than smoker periodontitis patients.

Key words: IgG2; immunoglobulin; periodontal pathogens; periodontitis; smoking

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A primary risk factor for periodontitis is dental plaque; specifically, a number of Gram-negative bacteria including, among others, *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*, have been implicated. Several studies have investigated specific antibody serum levels in relation to the aforementioned periodontal pathogens (Ebersole 1990, Lamster et al. 1990, Chen et al. 1991, Kinane et al. 1993, Albandar et al. 2001); however, sporadic information is available regarding

the total amounts of circulating antibody classes and subclasses, including immunoglobulin G (IgG) (and IgG subclasses), IgA and IgM in untreated periodontitis patients compared with controls (Gmür 1986, Ebersole et al. 1991, Kinane et al. 1993, Sakai et al. 2001). Similarly, knowledge on the total amounts of plasma Ig's in subjects culture positive or culture negative for *A. actinomycetemcomitans* or *P. gingivalis* in patients is unknown.

Several host-related determinants and environmental factors have been suggested to decrease antibody production or modify antibody production differentially. Both the periodontal and medical literature indicate that race, age, insulin-independent diabetes and smoking may be important in relation to variation in antibody production (Gulsvik & Fagerhoi 1979, Shackelford et al. 1985, Popa et al. 1993, Quinn et al. 1996, Smith et al. 1996, Tangada et al. 1997, Mooney et al. 2001, Moszczynski

et al. 2001, Qvarfordt et al. 2001). In particular, smoking has been associated with lower IgG2 levels and subjects of African-American heritage show higher levels of IgG2 compared with whites (Quinn et al. 1996, 1998, Gunsolley et al. 1997). It has been shown in the USA that Caucasian periodontitis patients who smoke have depressed levels of IgG2 in contrast to smoking black patients, who had comparable levels to control subjects without disease (Quinn et al. 1996, 1998, Gunsolley et al. 1997). Therefore, one of the possible biological explanations for the epidemiological associations between race, age, insulin-independent diabetes, smoking and periodontitis, may be related to modification of antibody production. It could be hypothesized that the lack of elevation of Ig's by aforementioned factors may result in less antibody protection in periodontitis, which in turn may increase disease severity.

The purpose of the present study was to investigate the effect of periodontal pathogens, smoking and type of periodontitis on the relative amounts of circulating total IgG and subclasses, IgA and IgM in European Caucasian subjects suffering from moderate and severe periodontitis in comparison with controls.

## Material and Methods

### Study population

Initially, a total of 168 subjects of Caucasian European heritage and >21 years were enrolled in this study. The study population included a consecutive series of untreated patients who were referred by their general dentist to the Department of Periodontology at ACTA for diagnosis and treatment of periodontitis, and control subjects who were registered for restorative dental procedures or regular dental check-ups. Only control subjects with  $\leq 1$  tooth per quadrant missing (third molars excluded) and who had at all approximal sites a radiographic distance between the cemento-enamel junction and the alveolar bone crest  $\leq 2$  mm on bite-wings  $\leq 1$ -year old, were included in the study. All subjects were both verbally and written informed about the purpose of the study. This study was approved by the Medical Ethical Committee of the Academic Medical Center of the University of Amsterdam.

On the basis of an extensive medical history by a written questionnaire and

by interview of 20–30 min duration, 33 subjects (20%) were excluded from the study for any of the following reasons: (1) taking chronically or in the last 2 weeks any type of medication (excluding birth control pills), (2) pregnancy, (3) apart from periodontitis, suffering from any given chronic medical condition, including diabetes, viral, fungal or bacterial infections, (4) suffering in the last 2 weeks from any medical condition, including flu, upper respiratory infection, allergy, skin disorders, sinus problem, (5) suffering in the last 2 weeks from any form of physical trauma, and (6) having had any type of dental work or tooth extraction(s) in the last 2 weeks. Thus, in total 135 subjects participated in the current study; these included 80 patients and 55 controls.

For all participants, (former) smoking habits were recorded; smokers were defined as those subjects who are current smokers ( $n = 53$ ) or who quit smoking in the previous 6 months ( $n = 9$ ) (Xu et al. 2002).

For each periodontitis patient, a set of full-mouth radiographs was available and the individual number of teeth present was determined. For each patient, all teeth were radiographically examined on the mesial and distal aspects. The number of teeth without bone loss, with bone loss extending beyond 1/3 of the root length and teeth with bone loss  $\geq 50\%$  of the root length was determined. Patients with  $\geq 7$  teeth with  $\geq 50\%$  bone loss were classified as having severe periodontitis ( $n = 29$ ) (adapted from Kornman et al. (1997)); the remainder of the patients was assigned to the group as having moderate periodontitis ( $n = 51$ ).

For some exploratory analyses, we divided all patients into two groups on the basis of diagnosis according to the current classification of periodontal diseases (Armitage 1999): (i) aggressive periodontitis (AgP) ( $n = 18$ ) or (ii) chronic periodontitis (CP) ( $n = 62$ ).

### Bacteriological samples

From all subjects, a pooled subgingival plaque sample was taken to assess the prevalence and the proportions of the following bacteria: *A. actinomycetemcomitans*, *P. gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Bacteroides forsythus*, *Peptostreptococcus micros* and *Campylobacter rectus*. In the periodontitis patients, sampling procedure was performed as described

previously (van Winkelhoff et al. 2002). In brief, pooled subgingival samples were taken from the deepest bleeding site per quadrant. In control subjects, samples were taken from the mesio- and distobuccal sites of the first molars; when these were not present, the second molars were used for sampling. The culturing and identification procedures of the samples were performed according to previously described standard procedures (van Winkelhoff et al. 1985). The detection level for *A. actinomycetemcomitans* is 10 cells/ml and for all other target species  $10^4$  cells/ml.

### Venous blood samples

Venous blood samples were obtained between 08:30 and 11:30 hours by venipuncture in the antecubital fossa without excessive venous stasis. The blood samples were obtained using EDTA-containing vacuum tubes (Venject II, Terumo Europe BV, Leuven, Belgium). The tubes were then centrifuged at 3000 r.p.m. for 10 min. The obtained plasma samples were stored in aliquots at  $-80^\circ\text{C}$  until use. The plasma levels of IgG, IgA and IgM were determined in a BNA-nephelometer with N Protein Standard SL for generation of standard curves (Dade Behring, Leusden, the Netherlands). Plasma levels of IgG subclasses were determined with commercially obtained ELISA kits (Sanquin, Amsterdam, the Netherlands) in microplate assays according to the manufacturer's instructions and using the standards provided with each kit. Results were analyzed with a Bio-Rad microplate reader and software (Bio-Rad, Hercules, CA, USA).

### Statistical analysis

Statistical analysis of data was performed with the SPSS package version 9.0 (SPSS, Chicago, IL, USA). Differences in frequencies for gender and smoking and prevalence of periodontopathogens among three groups of subjects (control, moderate periodontitis and severe periodontitis) were analyzed by Mantel-Haenszel tests. Differences between the three study groups for all variables were determined with analyses of covariance (ANCOVA) with periodontal disease status as a factor, while the potential confounders age, gender and smoking were also entered in the model as covariates. Unstandardized residuals of each of these ANCOVAs were tested for

their normal distribution in  $Q-Q$  plots. A non-normal distribution was found for IgM, IgG3 and 4. Subsequently observed values were log transformed and used for statistical analyses. However, IgG4-transformed values were still not distributed in a normal fashion. Therefore, a difference between the three groups for IgG4 was tested non-parametrically (Kruskal–Wallis test). Means and standard deviations for the different parameters were calculated. When overall ANCOVA showed statistical significance, post hoc testing was performed to explore the differences between any two groups. To illustrate differences between subgroups of subjects, box plots were constructed with the SPSS program and differences between subgroups were explored also using ANCOVA as explained above.  $p$ -values  $<0.05$  were considered significant.

## Results

Subject characteristics are presented in Table 1. The mean age of both control subjects and periodontitis patients was 43 years. There were more smokers among patients (severe and moderate periodontitis) than among controls ( $p = 0.007$ ). The three study groups

had a comparable number of teeth present. Patients with moderate periodontitis had on average 2.9 teeth with  $\geq 50\%$  bone loss, whereas the corresponding value was 14.3 for the patients with severe periodontitis (Table 1).

The results of the analyses of the subgingival plaque samples are presented in Table 2. A significant higher prevalence of the periodontopathogens *P. gingivalis*, *P. intermedia*, *B. forsythus* and *P. micros* in moderate and severe periodontitis versus control was observed. The proportion of target species in culture-positive subjects were not different among the three groups, except for *B. forsythus* ( $p = 0.049$ ).

In Table 3, mean plasma levels for total IgG, IgA and IgM are presented. Total IgG in the control group was 9.6 g/l, while corresponding values for moderate and severe periodontitis were 10.2 and 10.0 g/l, respectively. The overall analysis indicated that the levels of total IgG did not differ between the three study groups ( $p = 0.24$ ); in this analysis, smoking was a significant covariate associated with lower levels ( $p < 0.001$ ), while the covariates age and gender were not significant. Mean plasma levels of IgA did not differ between the three groups, but age was a sig-

nificant covariate ( $p = 0.003$ ) showing a positive correlation with IgA levels. Plasma levels of IgM showed no intergroup differences; none of the covariates included in the analysis was significant in the overall IgM analysis (Table 3).

Differentiation of total IgG into IgG isotypes was also studied (Table 4). In the overall analysis, it was seen that IgG1 plasma levels were significantly different between the three study groups. In this analysis, none of the potential confounders (age, gender, smoking and education) was significant. Post hoc testing between any two groups for IgG1 levels revealed higher IgG1 levels in severe periodontitis than controls, while the other paired comparisons were not significant. Plasma levels of IgG2 were significantly different between the three groups in the overall analysis; for this isotype, smoking was a significant covariate associated with lower levels. Post hoc testing showed that the severe and moderate periodontitis groups had higher levels than the controls. Plasma levels of IgG3 and IgG4 were both low (3–6 % of total IgG) and were not different between the three groups (Table 4). Also none of the covariates showed statistical significance in the overall analyses for IgG3.

Levels of Ig's were analyzed in relation to individual periodontal pathogens for the whole-study population. In these ANCOVAs being culture positive or negative was entered as factor, while periodontal status (control or patient), smoking, age and gender were entered as covariates. Subjects either culture positive or culture negative for *A. actinomycetemcomitans* ( $n = 24$  or  $n = 111$ , respectively), *P. gingivalis* ( $n = 53$  or  $n = 82$ , respectively), *P. intermedia* ( $n = 101$  or  $n = 34$ , respectively), *B. forsythus* ( $n = 95$  or  $n = 40$ , respectively), *P. micros* ( $n = 117$  or  $n = 18$ ,

Table 1. Characteristics for three study groups (control, moderate periodontitis and severe periodontitis)

	Control ( $n = 55$ )	Moderate ( $n = 51$ )	Severe ( $n = 29$ )
Age (years)	43.2 $\pm$ 12.6	43.7 $\pm$ 11.5	43.0 $\pm$ 8.8
Female (%)	53	63	52
Smoker (%)	35	47	66*
Number of teeth	27.8 $\pm$ 1.8	26.5 $\pm$ 3.4	26.5 $\pm$ 2.4
Teeth with no bone loss	27.8 $\pm$ 1.8	9.8 $\pm$ 7.2	2.5 $\pm$ 3.4
Teeth $> 1/3$ bone loss	0	5.9 $\pm$ 4.3	16.8 $\pm$ 5.1
Teeth $\geq 50\%$ bone loss	0	2.9 $\pm$ 1.8	14.3 $\pm$ 6.3

Values are means  $\pm$  standard deviation or % of subjects.

\*Frequency per group different at  $p < 0.01$  (Mantel–Haentzsel test).

Table 2. Results of the analyses of the subgingival microbial samples for control, moderate periodontitis and severe periodontitis

	Control ( $n = 55$ )		Moderate ( $n = 51$ )		Severe ( $n = 29$ )		$p$ -values
	$n$ (%)	proportions	$n$ (%)	proportions	$n$ (%)	proportions	
<i>Actinobacillus actinomycetemcomitans</i>	6 (11)	3.1 $\pm$ 4.6	12 (24)	2.4 $\pm$ 4.9	6 (21)	0.6 $\pm$ 0.6	0.175
<i>Porphyromonas gingivalis</i>	4 (7)	29.2 $\pm$ 31.3	34 (67)	27.3 $\pm$ 20.5	15 (52)	28.6 $\pm$ 20.6	$<0.001$
<i>Prevotella intermedia</i>	32 (58)	7.8 $\pm$ 10.4	43 (84)	5.1 $\pm$ 5.6	26 (90)	5.7 $\pm$ 7.1	0.001
<i>Bacteroides forsythus</i>	23 (42)	3.8 $\pm$ 3.7	46 (90)	8.8 $\pm$ 8.1	26 (90)	8.0 $\pm$ 10.5	$<0.001$
<i>F. nucleatum</i>	44 (80)	4.2 $\pm$ 3.3	46 (90)	6.7 $\pm$ 7.4	27 (93)	4.2 $\pm$ 4.3	0.068
<i>Peptostreptococcus micros</i>	38 (70)	5.5 $\pm$ 7.6	44 (86)	5.2 $\pm$ 6.9	26 (90)	4.5 $\pm$ 3.9	0.014

The left column per group represents prevalence, i.e. number ( $n$ ) of subjects culture positive (in parentheses % of subjects), while the right column per group represents the mean values for proportion  $\pm$  standard deviation of the microorganism of the cultivable anaerobic microflora in culture-positive subjects.

\* $p$ -values from Mantel–Haentzsel tests, testing differences in prevalence among the three study groups.

Table 3. Plasma levels (means ± standard deviations) of immunoglobulin (Ig) G, A and M in g/l in controls and patients with moderate and severe periodontitis

g/l	Control (n = 55)	Moderate (n = 51)	Severe (n = 29)	p-value
Total IgG*	9.6 ± 2.0	10.2 ± 2.5	10.0 ± 2.3	0.084 <sup>†</sup>
Total IgA*	2.1 ± 1.0	2.1 ± 0.9	2.2 ± 1.0	0.756 <sup>‡</sup>
Total IgM*	1.4 ± 0.7	1.6 ± 1.0	1.6 ± 0.9	0.327 <sup>§</sup>

\*The Academic Medical Center applies the following ranges of reference values for clinical diagnostic purposes: total IgG, 7.0–16.0 g/l; total IgA, 0.7–4.0 g/l; total IgM, 0.4–2.3 g/l.

<sup>†</sup>Significant covariate in the overall analysis of covariance (ANCOVA) was smoking (p < 0.001).

<sup>‡</sup>Significant covariate in the overall ANCOVA was age (p = 0.001).

<sup>§</sup>ANCOVA based on log-transformed values for IgM.

Table 4. Plasma levels (means ± standard deviations) of immunoglobulin (Ig) IgG1, IgG2, IgG3 and IgG4 in g/l in controls and patients with moderate periodontitis and severe periodontitis

g/l	Control (n = 55)	Moderate (n = 51)	Severe (n = 29)	p-value
IgG1*	4.2 ± 2.5	5.1 ± 2.0	6.0 ± 3.1 <sup>†</sup>	0.009
IgG2*	2.7 ± 1.3	3.4 ± 1.6 <sup>‡</sup>	3.7 ± 1.8 <sup>§</sup>	0.002 <sup>¶</sup>
IgG3*	0.3 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.151 <sup>  </sup>
IgG4*	0.4 ± 0.4	0.6 ± 0.8	0.5 ± 0.6	0.178 <sup>**</sup>

\*The manufacturer of the ELISA kits (Sanquin, Amsterdam, the Netherlands) provided the following information regarding reference values for clinical diagnostic purposes: IgG1, IgG2, IgG3 and IgG4 are normally present as 65%, 25%, 6% and 4% of total IgG, respectively.

<sup>†</sup>Post hoc testing between any two groups showed that plasma levels of IgG1 in severe periodontitis were higher than in control (p = 0.013).

<sup>‡</sup>Post hoc testing between any two groups showed that plasma levels of IgG2 in moderate were higher than in control (p = 0.013).

<sup>§</sup>Post hoc testing between any two groups showed that plasma levels of IgG2 in severe periodontitis were higher than in control (p = 0.001).

<sup>¶</sup>Significant covariate in the overall analysis of covariance (ANCOVA) was smoking (p = 0.009).

<sup>||</sup> ANCOVA based on log-transformed values for IgG3.

\*\*Kruskal–Wallis test since neither the residuals of untransformed IgG4 nor log-transformed values of IgG4 showed a normal distribution in Q–Q plots.

respectively) and *F. nucleatum* (n = 108 or n = 27, respectively) did not show differences in plasma levels of total IgG, IgA, IgM or IgG isotypes. However, in the great majority of these analyses, periodontal status and smoking were, as expected, significant covariates associated with higher and lower levels, respectively, for total IgG, IgG1 and for IgG2.

In the present study, smoking appeared a major factor. Smoker periodontitis patients had fewer teeth without bone loss compared with non-smoking patients: 5.1 and 9.6, respectively (Fig. 1). The number of teeth with bone loss beyond 1/3 of the root length was higher in the smoking patients than in non-smokers: 11.5 and 7.8, respectively. The mean number of teeth with ≥50% bone loss in smoking patients was 8.1 compared with a corresponding value of 5.8 in non-smoking patients; this difference was not significant (Fig. 1). In light of these differences in severity of periodontal breakdown, the effects of smoking on plasma IgG levels were further explored. Non-smoking patients showed

in the overall analyses, higher total IgG levels than non-smoking controls (Fig. 2A); in post hoc comparisons among the non-smokers, patients with moderate periodontitis had higher levels of total IgG than the controls. Also for non-smoking patients, significant increases compared with non-smoking controls were seen for IgG1 and for IgG2, with the severe non-smoker patients showing the highest levels (Figs 2B and C). In contrast, smokers with periodontitis show on average minimal or no elevation of total IgG (Fig. 2A) and IgG 2 (Fig. 2C), compared with smoker controls. The total IgG and IgG2 levels in smokers with periodontitis are comparable with levels of smoker controls. Levels of IgG1 in the smoker periodontitis patients seemed to follow the same pattern as levels in non-smokers, but this apparent dose–response increase from control to severe periodontitis failed to reach significance (Fig. 2B). Moreover, we analyzed per severity category whether smokers have lower Ig levels than non-smokers (Fig. 2). This was the case for total

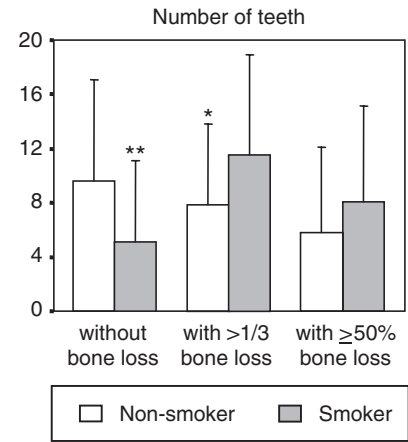


Fig. 1. Bar graphs representing mean ± standard deviation of numbers of teeth without alveolar bone loss, with bone loss > 1/3 of the root length and with bone loss ≥50% of the root length, for non-smoking (n = 43) and smoking periodontitis patients (n = 37). There was a significant difference between non-smokers and smokers for number of teeth without bone loss (\*\*p = 0.004) and for number of teeth with > 1/3 bone loss (\*p = 0.019).

IgG within the moderate periodontitis category (p = 0.004) and within the severe category (p = 0.046). In addition, smokers had lower levels of IgG2 within the severe category (p = 0.019)

IgG2 plasma levels in smoking and non-smoking periodontitis patients in relation to *A. actinomycetemcomitans* and *P. gingivalis* are shown in Fig. 3. Non-smoking patients being culture positive or negative for *A. actinomycetemcomitans*, showed comparable IgG2 levels. Also *A. actinomycetemcomitans* culture-negative and culture-positive smoker patients showed comparable IgG2 levels. However, again, levels of IgG2 in smokers were lower than in the non-smokers; this observation reached statistical significance for the *A. actinomycetemcomitans* culture-negative patients (p = 0.032). A similar IgG2 pattern was seen among the non-smoking and smoking patients with or without *P. gingivalis* in subgingival samples (Fig. 3B); non-smoking *P. gingivalis* culture-positive patients had higher levels of IgG2 than corresponding smoking patients (p = 0.019). Similar analyses among non-smoking and smoking patients were not performed for the other cultured microorganisms, as the numbers of patients culture-negative per subgroup were too low for meaningful exploration.

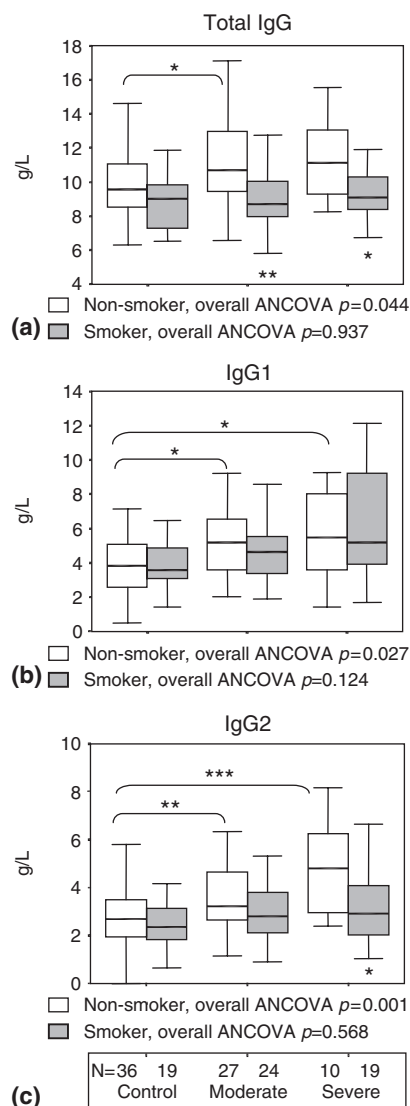


Fig. 2. Box plots for plasma levels of total IgG (panel A), IgG1 (panel B) and IgG2 (panel C) in non-smoking and smoking patients with AgP and CP. In AgP patients ( $n=18$ ), plasma levels of total IgG, IgG1 and IgG2 were comparable between non-smokers and smokers. However, in CP patients ( $n=67$ ), smokers showed lower levels of total IgG and IgG2 than non-smokers ( $p<0.01$  and  $p<0.001$ , respectively). Interestingly, between non-smoker AgP

and non-smoker CP, there were no differences for total IgG, IgG1 and IgG2 levels. Similarly, between smoking AgP and smoking CP no differences for the antibody outcome variables were observed.

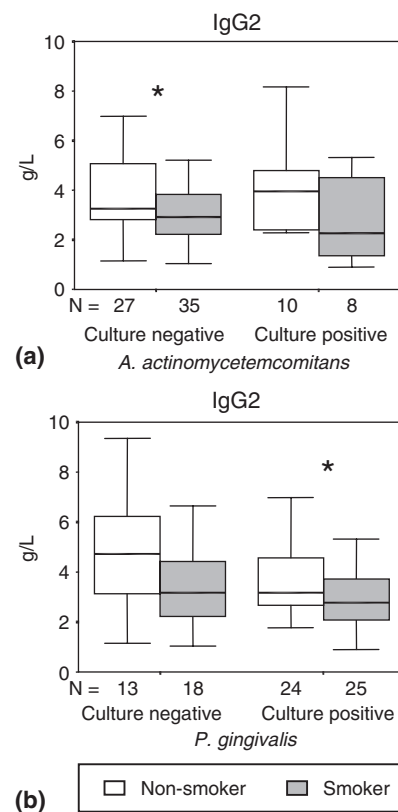


Fig. 3. Box plots for plasma levels IgG2 in non-smoking and smoking patients being either culture negative or culture positive for *Actinobacillus actinomycetemcomitans* (panel a) or *Porphyromonas gingivalis* (panel b). \* $p<0.05$ .

and non-smoker CP, there were no differences for total IgG, IgG1 and IgG2 levels. Similarly, between smoking AgP and smoking CP no differences for the antibody outcome variables were observed.

## Discussion

The major findings of the present study can be summarized as follows:

- In periodontitis, higher levels of IgG1 and IgG2 were observed compared with periodontally intact control subjects.
- Smoking was strongly associated with no or minimal rise in plasma levels of total IgG and IgG2 in periodontitis, despite the fact that the prevalence of several periodontal pathogens was increased.
- The elevation of total IgG, IgG1 and IgG2 in non-smoking periodontitis patients was increasing

with severity, but independent of whether patients were infected with the periodontal pathogens *A. actinomycetemcomitans* or *P. gingivalis* and independent of whether the disease was of the aggressive or chronic form.

- The lack of an elevated total IgG, IgG1 and IgG2 in smoking patients was irrespective of severity of the disease, infection with *A. actinomycetemcomitans* or *P. gingivalis*, or diagnosis.
- IgA and IgM levels were not different between periodontitis patients and controls, suggesting that IgA and IgM do not play a major role in periodontitis.

The current findings corroborate the observation by others (Wilton et al. 1992, Gunsolley et al. 1997, Quinn et al. 1998), that in periodontitis one can expect a rise in systemic IgG levels, but that smoking periodontitis patients do not show an elevation of systemic IgG levels, in particular IgG2. Similar to Quinn et al. (1998), we also find that smoking does not affect levels of IgG1, IgG3 and IgG4. In addition, we corroborate that in periodontitis levels of IgA and IgM are not elevated (Gmür 1986). The present values for total IgG, IgA and IgM and for IgG isotypes were all within the "normal" reference range reported by the literature. However, it should be noted that these reference values are obtained in a mixed population of smokers and non-smokers, and most likely that "normal" reference populations are mixed with respect to periodontal conditions.

Plasma levels of antibody are regarded indices of immunity and smoking is now an accepted risk factor for reduced levels of IgG (Moszczynski et al. 2001). Also in several other medical conditions, smoking has been shown to correlate negatively with plasma antibody levels, for example, in smokers with chronic bronchitis, with peptic ulcers and with recurrent respiratory infections (Popa et al. 1993, Calvet et al. 1997, Ogihara et al. 2000, Qvarfordt et al. 2001).

The major antibody response in periodontitis is IgG2, presumably in response to the bacterial polysaccharides and outer membrane proteins of Gram-negative periodontal pathogens (Schenck & Michaelsen 1987, Ebersole 1990, Mooney & Kinane 1994, Ogawa et al. 1994, Lamster et al. 1998). In

general, higher plasma levels of IgG2, especially reactive with *A. actinomycetemcomitans*, are considered as protective to periodontal breakdown (Gunsolley et al. 1990); however are they really protective (Albandar et al. 2001)? It seems surprising that systemic IgG2 levels in periodontitis show such a modest increase (Table 4), while IgG2 is considered the major antibody response to Gram-negative infectious diseases. The "normal" amount of IgG2 circulating in plasma ( $2.7 \pm 1.3$  g/l in the control group) perhaps should be regarded as natural or "surveilling" antibody with low specificity for periodontal pathogens. In fact, present circulating levels of IgG, IgA and IgM as well as the levels for IgG isotypes were unrelated to the culture results for *A. actinomycetemcomitans* and *P. gingivalis*. This may indicate that the reported *A. actinomycetemcomitans*- and *P. gingivalis*-specific antibodies for periodontitis patients represent actually a small part of the total antibody pool. Also, it indicates that many other microorganisms in addition to *A. actinomycetemcomitans* and *P. gingivalis*, elicit antibody responses in periodontitis (Albandar et al. 2001). Similarly, the modest increase of IgG1 in periodontitis (Table 4), independent of microbiological culture results, raises the question whether the periodontal infection causes a robust antibody response. In fact, it has been postulated that periodontitis-susceptible patients are characterized by the production of non-protective antibodies (Gemmell et al. 2002). On the other hand, it needs to be borne in mind that the systemic Ig levels may be different from locally produced Ig amounts. However, some studies showed correlations between systemic and local levels of IgG (Choi et al. 1990, Kinane et al. 1993).

The results of the present study and many others show that smoking patients in comparison with non-smoker patients have more severe periodontal bone loss. Therefore, an explanation for these findings could be that in smokers with periodontitis, a lack of sufficient protective antibodies (in particular IgG2 against Gram-negative microorganisms) is perhaps in part responsible for increased severity of destructive periodontal disease. The pathway by which smoking affects Ig production and, consequently, the pathogenesis of periodontitis is not understood. In general, in periodontitis a type 2 T-cell response

is occurring (reviewed by (Gemmell et al. 2002)) and this type of T-cell response is stimulatory for B-cells. However, IgG2 production in mice is enhanced by a type 1 T-cell response (Finkelman et al. 1988, Stevens et al. 1988). Other infectious or inflammatory conditions with a type 1 T-cell response, including *Lyme borreliosis*, tuberculosis and type 1 diabetes, have also shown an immune response with elevated levels of IgG1 without concomitant elevation of IgG2 (Sousa et al. 1998, Widhe et al. 1998, Bonifacio et al. 1999, Ng et al. 1999). Thus, a reduced IgG2 in smokers may also indicate that the balance between a type 1 and type 2 T-cell response in smokers is tipping more towards type 2, while in non-smokers a predominant type 1 T-cell activity is present. However, for the latter hypothesis no data are available. An alternative explanation is that smoking in general reduces T-cell responsiveness, resulting in a decrease of T-cell proliferation and T-cell-dependent antibody responses (Geng et al. 1995, 1996). The mechanisms whereby smoking affects T-cells are unknown. A third hypothesis regarding the difference in IgG2 response between non-smokers and smokers, involves cells of the monocyte/macrophage lineage. These cells may be less activated in smokers; for example, monocytes isolated from lung alveoli in smokers show depressed activity with respect to the secretion of IL-6, IL-1 $\beta$  and TNF- $\alpha$  (Soliman & Twigg 1992, Sauty et al. 1994). These latter cytokines stimulate B-cell proliferation, which in turn produce Ig's.

Quitting smoking seems to be beneficial for increasing systemic levels of antibody. There were 13 subjects within the non-smokers who quit smoking >6 months and <10 years before entering the study. The subjects had levels of total IgG and IgG2 in between the non-smokers and smokers. But these observations need to be interpreted with care as the number of former smokers is limited and subgroup analysis was not performed.

In conclusion, among Caucasian European subjects, smoker periodontitis patients fail to mount an elevated IgG2 response. This lack of increase in plasma IgG2 levels coincides with increased severity of periodontal destruction in the smoker patients. These findings are irrespective of whether subjects were infected with *A. actinomycetemcomitans* or *P. gingivalis*, and

irrespective of disease diagnosis. The current results identify another possible immunological factor that may explain more periodontal breakdown in smokers with periodontitis.

## References

- Albandar, J. M., DeNardin, A. M., Adesanya, M. R., Diehl, S. R. & Winn, D. M. (2001) Associations between serum antibody levels to periodontal pathogens and early-onset periodontitis. *Journal of Periodontology* **72**, 1463–1469.
- Armitage, G. C. (1999) Development of a classification system for periodontal diseases and conditions. *Annals of Periodontology* **4**, 1–6.
- Bonifacio, E., Scirpoli, M., Kredel, K., Fuchtenbusch, M. & Ziegler, A. G. (1999) Early autoantibody responses in prediabetes are IgG1 dominated and suggest antigen-specific regulation. *Journal of Immunology* **163**, 525–532.
- Calvet, X., Navarro, M., Gil, M., Mas, P., Rivero, E., Sanfeliu, I., Brullet, E., Campo, R., Dalmau, B. & Lafont, A. (1997) Seroprevalence and epidemiology of *Helicobacter pylori* infection in patients with cirrhosis. *Journal of Hepatology* **26**, 1249–1254.
- Chen, H. A., Johnson, B. D., Sims, T. J., Darveau, R. P., Moncla, B. J., Whitney, C. W., Engel, D. & Page, R. C. (1991) Humoral immune responses to *Porphyromonas gingivalis* before and following therapy in rapidly progressive periodontitis patients. *Journal of Periodontology* **62**, 781–791.
- Choi, J. I., Nakagawa, T., Yamada, S., Takazoe, I. & Okuda, K. (1990) Clinical, microbiological and immunological studies on recurrent periodontal disease. *Journal of Clinical Periodontology* **17**, 426–434.
- Ebersole, J. L. (1990) Systemic humoral immune responses in periodontal disease. *Critical Reviews in Oral Biology and Medicine* **1**, 283–331.
- Ebersole, J. L., Sandoval, M. N., Steffen, M. J. & Cappelli, D. (1991) Serum antibody in *Actinobacillus actinomycetemcomitans*-infected patients with periodontal disease. *Infection and Immunity* **59**, 1795–1802.
- Finkelman, F. D., Katona, I. M., Mosmann, T. R. & Coffman, R. L. (1988) IFN-gamma regulates the isotypes of Ig secreted during in vivo humoral immune responses. *Journal of Immunology* **140**, 1022–1027.
- Gemmell, E., Yamazaki, K. & Seymour, G. J. (2002) Destructive periodontitis lesions are determined by the nature of the lymphocytic response. *Critical Reviews in Oral Biology and Medicine* **13**, 17–34.
- Geng, Y., Savage, S. M., Johnson, L. J., Seagrave, J. & Sopori, M. L. (1995) Effects of nicotine on the immune response. I. Chronic exposure to nicotine impairs antigen receptor-mediated signal transduction in lymphocytes. *Toxicology and Applied Pharmacology* **135**, 268–278.

- Geng, Y., Savage, S. M., Razani-Boroujerdi, S. & Sopori, M. L. (1996) Effects of nicotine on the immune response. II. Chronic nicotine treatment induces T cell anergy. *Journal of Immunology* **156**, 2384–2390.
- Gmür, R. (1986) Double blind analysis of the relation between adult periodontitis and systemic host response to suspected periodontal pathogens. *Infection and Immunity* **52**, 768–776.
- Gulsvik, A. & Fagerhoi, M. K. (1979) Smoking and immunoglobulin levels. *The Lancet* **1**, 449.
- Gunsolley, J. C., Pandey, J. P., Quinn, S. M., Tew, J. & Schenkein, H. A. (1997) The effect of race, smoking and immunoglobulin allotypes on IgG subclass concentrations. *Journal of Periodontal Research* **32**, 381–387.
- Gunsolley, J. C., Tew, J. G., Gooss, C., Marshall, D. R., Burmeister, J. A. & Schenkein, H. A. (1990) Serum antibodies to periodontal bacteria. *Journal of Periodontology* **61**, 412–419.
- Kinane, D. F., Mooney, J., MacFarlane, T. W. & McDonald, M. (1993) Local and systemic antibody response to putative periodontopathogens in patients with chronic periodontitis: correlation with clinical indices. *Oral Microbiology and Immunology* **8**, 65–68.
- Kornman, K. S., Crane, A., Wang, H. Y., diGiovine, F. S., Newman, M. G., Pirk, F. W., Wilson, T. G. Jr., Higginbottom, F. L. & Duff, G. W. (1997) The interleukin-1 genotype as a severity factor in adult periodontal disease. *Journal of Clinical Periodontology* **24**, 72–77.
- Lamster, I. B., Celenti, R. & Ebersole, J. L. (1990) The relationship of serum IgG antibody titers to periodontal pathogens to indicators of the host response in crevicular fluid. *Journal of Clinical Periodontology* **17**, 419–425.
- Lamster, I. B., Kaluszner-Shapira, I., Herrera-Abreu, M., Sinha, R. & Grbic, J. T. (1998) Serum IgG antibody response to *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*: implications for periodontal diagnosis. *Journal of Clinical Periodontology* **25**, 510–516.
- Mooney, J., Hodge, P. J. & Kinane, D. F. (2001) Humoral immune response in early-onset periodontitis: influence of smoking. *Journal of Periodontal Research* **36**, 227–232.
- Mooney, J. & Kinane, D. F. (1994) Humoral immune responses to *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* in adult periodontitis and rapidly progressive periodontitis. *Oral Microbiology and Immunology* **9**, 321–326.
- Moszczyński, P., Zabinski, Z., Moszczyński, P. Jr., Rutowski, J., Slowinski, S. & Tabarowski, Z. (2001) Immunological findings in cigarette smokers. *Toxicology Letters* **118**, 121–127.
- Ng, W. Y., Thai, A. C., Lui, K. F., Yeo, P. P. & Cheah, J. S. (1999) Systemic levels of cytokines and GAD-specific autoantibodies isotypes in Chinese IDDM patients. *Diabetes Research and Clinical Practice* **43**, 127–135.
- Ogawa, T., Ogo, H., Uchida, H. & Hamada, S. (1994) Humoral and cellular immune responses to the fimbriae of *Porphyromonas gingivalis* and their synthetic peptides. *Journal of Medical Microbiology* **40**, 397–402.
- Ogihara, A., Kikuchi, S., Hasegawa, A., Kurosawa, M., Miki, K., Kaneko, E. & Mizukoshi, H. (2000) Relationship between *Helicobacter pylori* infection and smoking and drinking habits. *Journal of Gastroenterology and Hepatology* **15**, 271–276.
- Popa, V., Kim, K. & Heiner, D. C. (1993) IgG deficiency in adults with recurrent respiratory infections. *Annals of Allergy* **70**, 418–424.
- Quinn, S. M., Zhang, J. B., Gunsolley, J. C., Schenkein, J. G., Schenkein, H. A. & Tew, J. G. (1996) Influence of smoking and race on immunoglobulin G subclass concentrations in early-onset periodontitis patients. *Infection and Immunity* **64**, 2500–2505.
- Quinn, S. M., Zhang, J. B., Gunsolley, J. C., Schenkein, H. A. & Tew, J. G. (1998) The influence of smoking and race on adult periodontitis and serum IgG2 levels. *Journal of Periodontology* **69**, 171–177.
- Qvarfordt, I., Riise, G. C., Andersson, B. A. & Larsson, S. (2001) IgG subclasses in smokers with chronic bronchitis and recurrent exacerbations. *Thorax* **56**, 445–449.
- Sakai, Y., Shimauchi, H., Ito, H. O., Kitamura, M. & Okada, H. (2001) *Porphyromonas gingivalis*-specific IgG subclass antibody levels as immunological risk indicators of periodontal bone loss. *Journal of Clinical Periodontology* **28**, 853–859.
- Sauty, A., Mauel, J., Philippeaux, M. M. & Leuenberger, P. (1994) Cytostatic activity of alveolar macrophages from smokers and nonsmokers: role of interleukin-1 beta, interleukin-6, and tumor necrosis factor-alpha. *American Journal of Respiratory Cell and Molecular Biology* **11**, 631–637.
- Schenck, K. & Michaelsen, T. E. (1987) IgG subclass distribution of serum antibodies against lipopolysaccharide from *Bacteroides gingivalis* in periodontal health and disease. *Acta Pathologica et Microbiologica Scandinavica* **95**, 41–46.
- Shackelford, P. G., Granoff, D. M., Nahm, M. H., Scott, M. G., Suarez, B., Pandey, J. P. & Nelson, S. J. (1985) Relation of age, race, and allotype to immunoglobulin subclass concentrations. *Pediatric Research* **19**, 846–849.
- Smith, G., Greenbaum, C., Johnson, B. & Persson, G. (1996) Short-term responses to periodontal therapy in insulin-dependent diabetic patients. *Journal of Periodontology* **67**, 794–802.
- Soliman, D. M. & Twigg, H. L. III (1992) Cigarette smoking decreases bioactive interleukin-6 secretion by alveolar macrophages. *American Journal of Physiology* **263**, L471–L478.
- Sousa, A. O., Henry, S., Maroja, F. M., Lee, F. K., Brum, L., Singh, M., Lagrange, P. H. & Aucouturier, P. (1998) IgG subclass distribution of antibody responses to protein and polysaccharide mycobacterial antigens in leprosy and tuberculosis patients. *Clinical and Experimental Immunology* **111**, 48–55.
- Stevens, T. L., Bossie, A., Sanders, V. M., Fernandez-Botran, R., Coffman, R. L., Mosmann, T. R. & Vitetta, E. S. (1988) Regulation of antibody isotype secretion by subsets of antigen-specific helper T cells. *Nature* **334**, 255–258.
- Tangada, S., Califano, J., Nakashima, K., Quinn, S., Zhang, J., Gunsolley, J., Schenkein, H. & Tew, J. (1997) The effect of smoking on serum IgG2 reactive with *Actinobacillus actinomycetemcomitans* in early-onset periodontitis patients. *Journal of Periodontology* **68**, 842–850.
- van Winkelhoff, A. J., Carlee, A. W. & de Graaff, J. (1985) *Bacteroides endodontalis* and other black-pigmented *Bacteroides* species in odontogenic abscesses. *Infection and Immunity* **49**, 494–497.
- van Winkelhoff, A. J., Loos, B. G., van der Reijden, W. & van der Velden, U. (2002) *Porphyromonas gingivalis*, *Bacteroides forsythus* and other putative periodontal pathogens in subjects with and without periodontal destruction. *Journal of Clinical Periodontology* **29**, 1023–1028.
- Widhe, M., Ekerfelt, C., Forsberg, P., Bergstrom, S. & Ernerudh, J. (1998) IgG subclasses in *Lyme borreliosis*: a study of specific IgG subclass distribution in an interferon-gamma-predominated disease. *Scandinavian Journal of Immunology* **47**, 575–581.
- Wilton, J. M., Hurst, T. J., Sterne, J. A., Caves, J., Tilley, C. & Powell, J. R. (1992) Elevated levels of the IgG2 subclass in serum from patients with a history of destructive periodontal disease. A case-control study. *Journal of Clinical Periodontology* **19**, 318–321.
- Xu, L., Loos, B. G., Craandijk, J., Ritsema, E., Huffels, R. A. M. & Van der Velden, U. (2002) Teeth with periodontal bone loss, cigarette smoking and plasma cotinine levels. *Journal of the International Academy of Periodontology* **4**, 39–43.

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