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Influence of Smoking and Race on Immunoglobulin G Subclass Concentrations in Early-Onset Periodontitis Patients

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Recent data indicate that smoking is an important risk factor for the development of periodontitis. Smoking is also known to reduce serum immunoglobulin G (IgG) levels. Interestingly, patients with the localized form of early-onset periodontitis (LJP) have elevated levels of serum IgG2, and those who smoke are not clinically different from nonsmoking LJP subjects. In contrast, patients with the generalized form of early-onset periodontitis (G-EOP) who smoke have more extensive destruction than their nonsmoking counterparts. Given the effects of smoking on EOP and the association of IgG2 with less severe disease, we hypothesized that smoking might reduce serum IgG2 and that this might be most apparent in G-EOP. We therefore examined the effects of smoking on serum IgG subclass concentrations in race-matched groups: LJP, G-EOP, and age-matched periodontally healthy controls (NPs). Smoking status was established from serum cotinine levels, and serum IgG subclass concentrations were determined by using radial immunodiffusion. The data indicated that the effects of smoking were remarkably selective with respect to both IgG subclass and race. Smoking did not appear to have any effect on the concentration of IgG1 or IgG3 in either black or white subjects. In contrast, smoking was associated with depressed serum IgG2 concentrations in both white NP and G-EOP subgroups. Serum IgG2 levels in black subjects did not appear to be depressed by smoking, with the single striking exception of the black G-EOP subgroup which also had depressed serum IgG4 levels. The results here confirm that smoking has effects on serum immunoglobulin levels, but the effects were both race and serum IgG subclass specific. Furthermore, the periodontal diagnosis of EOP subjects appeared to be important, as indicated by the fact that IgG2 and IgG4 levels were reduced in smoking black G-EOP subjects whereas the IgG2 and IgG4 levels in black LJP and NP subjects were not reduced by smoking.

Early-onset periodontitis (EOP) presents clinically in two forms. This key feature was used to classify EOP into two broad categories, localized (LJP) and generalized (G-EOP), in a recent EOP workshop (39). EOP develops between puberty and early adulthood, and the disease tends to aggregate in families. Interestingly, both LJP and G-EOP can be found within the same family. The clustering of this rare disease within families may be explainable by a gene, inherited in an autosomal dominant pattern, that renders subjects susceptible to disease (26, 33). The difference in clinical expression between the two forms likely relates to differences in microbial flora and differences in the ability to respond immunologically (5, 6, 8, 15, 31).

Recent data indicate that smoking is an important risk factor for the development of periodontitis. Smoking is associated with increased attachment loss and loss of supporting alveolar bone (2, 3, 11, 12, 16–19), and the prevalence of smoking may be very high in patients at risk for recurrent periodontitis (25). Interestingly, smoking is common among patients with G-EOP, and smoking G-EOP patients have significantly more affected teeth and greater mean levels of attachment loss than their nonsmoking counterparts. In marked contrast, smoking is much less frequent in LJP subjects and they appear to be resistant to smoking, i.e., smoking LJP patients are not clinically different from nonsmoking LJP patients (34).

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Previous studies also show that serum immunoglobulin G2 (IgG2) levels in LJP patients are higher than serum IgG2 levels in both G-EOP subjects and age-matched periodontally healthy controls (NPs) (24). IgG2 is the major Ig subclass that reacts with bacterial components such as carbohydrates and lipopolysaccharides (20, 30, 32, 35, 36, 38, 40), and it may be a good opsonin (23, 42). The level of IgG2 reactive with *Actinobacillus actinomycetemcomitans* is frequently more than 100 μg/ml in EOP patients (41) and seropositivity is associated with less severe periodontal disease (14). On this basis, it was reasoned that the elevated level of IgG2 in LJP patients may be protecting them from developing a more severe form of periodontitis. Studies also indicate that serum IgG2 levels are affected by race, with the levels in black subjects in all periodontal groups being higher than those of their white counterparts (24).

Given the effects of smoking on EOP and the association of IgG2 with less severe disease, we hypothesized that smoking might reduce serum IgG2 and that this might be most apparent in G-EOP. We therefore examined the effects of smoking, as assessed by serum cotinine concentrations, on serum IgG concentrations in race-matched groups: LJP, G-EOP, and NP. None of the serum IgG subclass concentrations of black LJP and NP subjects were affected by smoking. In contrast, the IgG2 concentrations of G-EOP subjects of both races were suppressed by smoking. Thus, the concentration of IgG2, which might be protective against periodontal infections, is decreased by smoking, and this may explain why smoking is more harmful in G-EOP subjects than in LJP subjects.

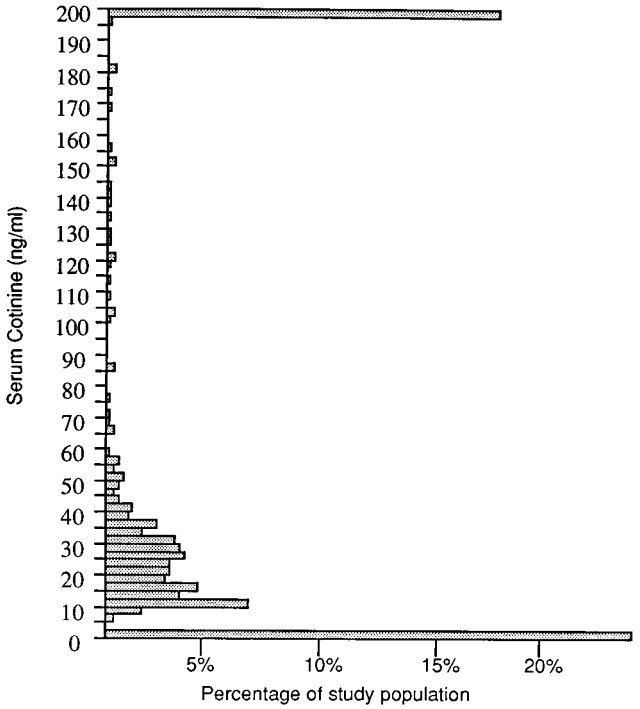

FIG. 1. Distribution of serum cotinine concentrations in the study population. The range of cotinine values varied from too low to measure (shown as 0) to 1,377 ng/ml. The grouping at the 200-ng/ml mark is a representation of all the individuals with cotinine levels between 200 and 1,377 ng/ml.

MATERIALS AND METHODS

Human subjects. Study subjects were obtained from the Clinical Research Center for Periodontal Diseases, School of Dentistry, Medical College of Virginia, Richmond, Va. Subjects who did not report their race to be black or white were excluded, since no other racial group was large enough to be evaluated.

Subjects were diagnosed as being periodontally healthy or as having one of two types of EOP, LJP or G-EOP. Patients with LJP were less than 30 years old and had a localized pattern of severe periodontal destruction limited to first molar or incisor teeth and up to two additional teeth. Patients with G-EOP were less than 35 years old and had a generalized pattern of severe destruction, with attachment loss of at least 5 mm on eight or more teeth, at least three of which were not first molars or incisors. Individuals classified as periodontally healthy (NPs) had no evidence of attachment loss, except for recession on the buccal surface of anterior teeth at no more than one site or pockets no greater than 3 mm.

Attachment loss measurements (from cemento-enamel junction to base of pocket) were made on all teeth with a Marquis probe at four places: mesiobuccal, distobuccal, midbuccal, and midlingual locations. Measurements were recorded to the nearest millimeter. Pocket depth, Silness and Löe plaque index (37), and Löe and Silness gingival index (22) were also measured at the same sites.

Sera. Blood samples were obtained by venipuncture. Sera were collected and stored at −80°C until the day before the assay, when it was removed from the freezer and allowed to thaw overnight at 4°C. Serum samples were diluted 1/10 for quantitation of IgG1 and IgG2 and were undiluted when tested for quantitation of IgG3, IgG4, and cotinine levels.

Cotinine assay. Exposure to tobacco products was assessed by measuring serum cotinine, a stable metabolite of nicotine, by ^{125}I radioimmunoassay. For this purpose, the commercially available Double Antibody Nicotine Metabolite Kits were purchased from Diagnostic Products Corporation (Los Angeles, Calif.) and the manufacturer's detailed instructions for quantitative analysis of cotinine levels were followed. The range of cotinine values varied from too low to measure (shown as 0 in Fig. 1) to 1,377 ng/ml. The grouping at the 200-ng/ml mark is a representation of all the individuals with cotinine levels between 200 and 1,377 ng/ml (Fig. 1). Note that approximately 20% of the population had cotinine levels of 200 ng/ml or greater. The distribution of serum cotinine concentrations in the study population was biphasic, with a large group of subjects exhibiting levels below 60 ng/ml. In our total data set, we have questionnaires from 238 subjects who indicated that they were nonsmokers. Twelve of these subjects had cotinine levels in excess of 300 ng/ml which is hard to reconcile with a nonsmoking status. To help establish a cutoff for smoking, we eliminated the 12 subjects with cotinine levels greater than 300 ng/ml, which left 95% of the subjects who reported that they were nonsmokers with cotinine levels below 75 ng/ml. When this cutoff value of 75 ng/ml was used to distinguish smokers from nonsmokers, the percentage of smokers in the study population was 25. This 25% compares favorably with the U.S. population which was 25% (95% confidence interval = ±0.7%) in 1993 (4). Cutoff levels of 50 or 100 ng/ml changed the status of only a small number of subjects and did not significantly alter the results.

Assay for Ig subclasses. Ig subclasses were measured by using radial immunodiffusion (RID) plates designed to specifically measure subclass concentrations. The RID plates were obtained from the Binding Site, Inc. (San Diego, Calif.) and used as recommended in the accompanying literature. Appropriately diluted calibrator, control, and patient samples were applied in 5-μl volume into wells on the RID plates containing monospecific antiserum in agarose gel. After complete diffusion, approximately 72 h, the ring diameters were measured to the nearest 0.1 mm independently by two people with no knowledge of the periodontal status of the subjects. The concentration of each test sample was calculated from the standard curve provided by the manufacturer of the RID plates. If the diffusion ring was outside the standard range, the serum samples were either diluted or reanalyzed at higher or lower dilutions.

Statistical analysis. Three-way analysis of variance was used to evaluate the effects of diagnosis, race, and smoking on cotinine levels. We have previously looked at the effects of race and diagnosis on IgG subclass levels. Therefore, the smoking effect was evaluated with an unpaired *t* test within each diagnosis group subdivided by race. For evaluation of the relationship between serum cotinine levels and IgG subclass levels, Spearman correlations were used for subjects subdivided by smokers and nonsmokers.

RESULTS

The demographic and clinical data for the various groups of young adults used in this study are listed in Table 1. Although there were large differences in periodontal status between the three groups, there were no substantial differences between the clinical values for the races within the three groups. Unfortunately, our group of white LJP subjects was small, but all of the other groups were available in adequate numbers.

To begin analysis, subjects who smoked had to be distinguished from those who did not smoke. As described in Materials and Methods, smoking was inferred from serum cotinine levels that exceeded 75 ng/ml. Data indicating the relationship between periodontal diagnosis, race, and the prev-

TABLE 1. Demographic and periodontal characteristics of patient groups[a]

Diagnosis	Race	No. of patients	Age (yr)	Plaque index	Gingival index	Pocket depth (mm)	Attachment loss[b]
NP	White	103	23.7 ± 0.6	0.53 ± 0.04	0.55 ± 0.04	2.01 ± 0.03	0.11 ± 0.01
	Black	82	20.4 ± 0.6	0.78 ± 0.05	0.79 ± 0.04	2.09 ± 0.03	0.07 ± 0.01
LJP	White	13	23.8 ± 2.1	0.98 ± 0.16	1.13 ± 0.12	2.61 ± 0.12	0.78 ± 0.08
	Black	72	21.1 ± 0.6	0.96 ± 0.04	1.07 ± 0.04	2.68 ± 0.05	0.70 ± 0.05
G-EOP	White	54	29.6 ± 0.96	1.31 ± 0.07	1.35 ± 0.06	3.39 ± 0.09	2.58 ± 0.16
	Black	66	28.4 ± 0.64	1.39 ± 0.06	1.39 ± 0.05	3.79 ± 0.11	2.51 ± 0.17

[a] An extensive comparative analysis of the demographic and periodontal characteristics of these populations, including the effect of smoking on the number of affected teeth and attachment loss, is presented in a recent publication by Schenkein et al. (34). All values given are means ± standard errors.

[b] Cemento-enamel junction to base of pocket.

TABLE 2. Periodontal status, race, and prevalence of smoking

Diagnosis	Race	Cotinine concn of >75 ng/ml	No. of patients	Mean cotinine concn (ng/ml) ± SE	% Smokers
NP	White	+	17	524 ± 26	16
		−	86	12.5 ± 1.8	
	Black	+	11	364 ± 73	13
		−	71	17.3 ± 1.8	
LJP	White	+	1	506.5	8
		−	12	18.3 ± 4.5	
	Black	+	13	284 ± 48	18
		−	59	18.9 ± 2.1	
G-EOP	White	+	26	423 ± 44	52
		−	28	22.9 ± 3.3	
	Black	+	27	418 ± 49	41
		−	39	19.7 ± 2.6	

alence of smoking are presented in Table 2. Note that the mean levels of serum cotinine in all smoking populations were similar, regardless of race or periodontal status. Similarly, all nonsmoking populations had comparable mean serum cotinine levels. Only one white LJP patient was identified as a smoker. Consequently, this subgroup was not large enough for analysis of the effect of smoking on IgG subclass concentrations.

Serum IgG2 levels are known to be related to race, with blacks exhibiting higher levels than whites (24). Consequently, each periodontal group (NP, LJP, and G-EOP) was divided by race and smoking status for analysis of serum subclass concentrations. The effects of smoking on the control group's (NP) IgG concentrations are summarized in Table 3. As expected, the concentration of each IgG subclass was higher in both the smoking and nonsmoking black subjects than in their white counterparts. Smoking did not appear to be associated with alteration of any serum subclass concentration, except for IgG2, where there was a 38% depression ($P < 0.002$) of IgG2 levels in white NPs. However, the concentration of serum cotinine did not correlate with the level of serum IgG2 depression in white NPs. Initial results were obtained using 25 to 30 people; additional subjects were subsequently added when a power calculation suggested that a smoking-induced alteration might exist. For each of the other IgG subclasses, there was a slight trend toward depression, but nothing that approached significance.

The serum IgG data for smoking and nonsmoking black LJP patients are recorded in Table 4. Smoking did not depress the concentration of any of the IgG subclasses. In fact, the trend in IgG1, IgG2, and IgG3 was toward higher levels in smokers.

The serum IgG subclass data for smoking and nonsmoking G-EOP patients are recorded in Table 5. Again, the concentration of each IgG subclass was higher in both the smoking and nonsmoking black patients than in their white counterparts. It appeared that smoking does not affect the serum IgG1 and IgG3 concentrations of white or black G-EOP patients. However, serum IgG2 levels were significantly lower for smoking white G-EOP patients, as they were for white NP subjects. However, in black G-EOP patients, both the IgG2 and IgG4 levels were decreased 20 to 30% by smoking. These effects in black subjects were unique to the G-EOP group. Again the concentration of serum cotinine did not correlate with the degree of IgG2 or IgG4 suppression in serum.

DISCUSSION

Smokers have been reported to be at increased risk for the development of periodontal disease (2, 3, 11, 12, 16–19). The average frequency of smoking in the U.S. population, as determined by the 1993 National Health Interview Survey for the Centers for Disease Control and Prevention was reported to be 25% (95% confidence interval = ±0.7%) (4). Subjects selected because they were periodontally healthy smoked less than the national average (about 15% smoked), while patients diagnosed with G-EOP smoked far more than the national average (nearly 50% smoked [Table 2]). The LJP group represents an apparent exception to the correlation between smoking and periodontal disease. These patients smoked no more frequently than the healthy controls but still had disease. Interestingly, smoking G-EOP subjects have more affected teeth and exhibit a greater mean attachment loss than nonsmoking G-EOP subjects (34). On the basis of the association of IgG2 with less severe disease, it was hypothesized that smoking might reduce serum IgG2 levels and that this might be most apparent in G-EOP subjects. The present study shows that smoking G-EOPs have significantly lower serum IgG2 levels than their nonsmoking counterparts. In marked contrast, patients with LJP did not appear to be affected by smoking. In fact, the trend was toward elevated levels of serum IgG2 in smokers, which correlates with the lack of any adverse clinical effects of smoking on the periodontal health of LJP patients (34).

Smoking has previously been reported to be associated with lower levels of serum IgG (1, 7, 9, 13, 21, 27). The present

TABLE 3. Effect of smoking on serum IgG subclass concentrations in young adults with a healthy periodontium

IgG subclass	White subjects				Black subjects			
	Smoking status	No. of subjects	Mean concn (μg/ml) ± SE	*P*	Smoking status	No. of subjects	Mean concn (μg/ml) ± SE	*P*
IgG1	+	26	5,557 ± 507	NS[a]	+	12	7,446 ± 816	NS
	−	72	6,411 ± 303		−	26	9,116 ± 669	
IgG2	+	17	1,983 ± 201	<0.002	+	11	3,838 ± 400	NS
	−	86	3,224 ± 170		−	71	3,736 ± 148	
IgG3	+	11	458 ± 52	NS	+	12	730 ± 89	NS
	−	13	660 ± 125		−	26	798 ± 77	
IgG4	+	11	267 ± 47	NS	+	12	546 ± 83	NS
	−	13	396 ± 56		−	26	649 ± 64	

[a] NS, not significant.

TABLE 4. Effect of smoking on serum IgG subclass concentrations in young black adults with LJP[a]

IgG subclass	Smoking status	No. of patients	Mean concn (μg/ml) ± SE	P
IgG1	+	15	10,240 ± 872	NS[b]
	−	40	9,256 ± 498	
IgG2	+	13	5,431 ± 508	NS
	−	59	5,038 ± 242	
IgG3	+	16	973 ± 101	NS
	−	42	930 ± 59	
IgG4	+	16	569 ± 89	NS
	−	42	603 ± 51	

[a] Since only one smoking white LJP patient was identified, this subgroup was not large enough for analysis.
[b] NS, not significant.

report confirms and extends this finding. Data in the present study indicated that the effects of smoking were selective with respect to effects on both IgG subclass and race. Smoking did not appear to have any effect on the concentration of IgG1 or IgG3 in either black or white subjects. In contrast, serum IgG2 concentrations were depressed in white NP and G-EOP groups. Serum IgG2 levels in black subjects did not appear to be depressed by smoking, with the single striking exception of the black G-EOP subgroup. Thus, in previous studies indicating that smoking reduced serum IgG levels, the patient population was primarily white and the reduction in serum IgG was likely attributable to a reduction in IgG2. Interestingly, Ig allotypes are significantly associated with Ig subclass concentrations in a race-specific manner (10). A recent study by Pandey et al. (29a) indicated that white subjects with Igs bearing the GM2 allotype were resistant to the effects of smoking. When white patients with the GM2 allotype were removed from the white G-EOP subset in this study, the significance of smoking was strengthened with a change in P value from $P < 0.04$ (Table 5) to $P < 0.001$. Similarly, removal of white GM2 allotype-positive people from the NP group also improved the statistical significance. We are currently examining the black G-EOP group for an Ig allotype which might help explain why their IgG2 levels are susceptible to the effects of smoking, while blacks in general are resistant. Interestingly, the serum IgG4 concentrations were also significantly depressed in black G-EOP smokers and Pandey et al. (29a) have found an Ig allotype effect on the level of IgG4 in blacks.

Our results and the results from numerous other groups indicate that smoking is associated with reduced serum IgG levels (1, 7, 9, 13, 21, 27). These results, however, conflict with the results of O'Keeffe et al. (29) and Merrill et al. (28). Both groups reported that total IgG concentrations were not suppressed by smoking. In addition, both O'Keeffe et al. and Merrill et al. examined serum IgG2 levels in a small group of subjects but did not find a significant depression associated with smoking. However, O'Keefe et al. did report that patients with chronic obstructive pulmonary diseases, diseases that are characteristically related to long-term cigarette use, had significantly lower levels of IgG2 than healthy controls. The reason their results differ from ours and others are not understood, but age and race could be important. For example, the average age of the O'Keeffe et al. study population was approximately 66, with a range of 50 to 80 years, while our study population consisted of young adults. This is important since serum IgG2 concentrations increase with age (24). Also, it appears that the O'Keeffe population was largely Irish and it is possible that the smokers in the study had an allotype that made them resistant to the effects of smoking. As for the Merrill et al. study, the racial makeup of the population is unknown.

We previously reported that LJP patients have serum IgG2 levels which are significantly higher than those in G-EOP patients or NP subjects (24). We reasoned that an ability to initiate a high IgG2 response might be protecting the LJP patients from developing the more severe G-EOP form of EOP. However, results from the present study show that when the large number of smoking G-EOP patients are removed from the data set, the level of IgG2 in nonsmoking G-EOP patients was about the same as that in the LJP patients (4,849 μg/ml for nonsmoking black G-EOP patients versus 5,038 μg/ml for nonsmoking black LJP patients [Tables 4 and 5]). Thus, it is difficult to attribute the difference in clinical status in these two nonsmoking groups solely to differences in the ability to make IgG2. However, the data reported here are consistent with the possibility that there could be a gene that regulates how smoking impacts the EOP patients. Smoking black G-EOP patients appear to be smoking susceptible, and this is associated with a more severe clinical condition and lower IgG2 levels. Meanwhile black LJP patients are smoking resistant, and their clinical conditions and serum IgG2 levels are unaffected.

Although smoking, as assessed by serum cotinine levels, was associated with alterations in serum IgG2 in both white NP

TABLE 5. Effect of smoking on serum IgG subclass concentrations in young adults with G-EOP

IgG subclass	White subjects				Black subjects			
	Smoking status	No. of subjects	Mean concn (μg/ml) ± SE	P	Smoking status	No. of subjects	Mean concn (μg/ml) ± SE	P
IgG1	+	17	6,259 ± 662	NS[a]	+	19	8,358 ± 764	NS
	−	16	6,116 ± 832		−	20	7,847 ± 620	
IgG2	+	26	2,858 ± 234	<0.04	+	27	3,437 ± 252	<0.0007
	−	28	3,581 ± 243		−	39	4,849 ± 338	
IgG3	+	17	720 ± 106	NS	+	19	1,039 ± 119	NS
	−	16	716 ± 82		−	20	912 ± 91	
IgG4	+	17	348 ± 78	NS	+	32	470 ± 51	<0.03
	−	16	275 ± 55		−	49	612 ± 41	

[a] NS, not significant.

subjects and white and black G-EOP patients, the effect of smoking did not appear to be quantitative. That is, those who had very high cotinine levels did not appear to be worse off than those with moderate levels of serum cotinine. It should be noted, however, that cotinine levels reflect only recent exposure to nicotine and not the long-term cumulative effects of smoking. Our LJP population is approximately 6 years younger than our G-EOP population. Since the LJP subjects are younger, they probably have not had the opportunity to smoke as much as the G-EOP subjects. Currently, a questionnaire is being developed to help determine if the number of years an individual smokes is related to their immunological response. An alternative explanation for the differences between G-EOP and LJP patients may relate to whether periodontal disease is active. The onset of LJP is believed to be around puberty and frequently shows a burnout phenomenon by adulthood. Since many of our LJP subjects are in their early twenties, they may no longer have active disease, and therefore their responses may be similar to healthy controls. In contrast, the G-EOP patients may have active disease and thus their responses remain sensitive to the effects of smoking.

Although smoking seems to affect LJP and G-EOP patients differently, it does not appear that this indicates a fundamental difference in the way these EOP patients respond to the same bacterial infection. In a recent study by Tangada et al. (38a), it appears that the level of serum antibody reactive with *A. actinomycetemcomitans* in nonsmoking LJP and G-EOP patients is indistinguishable. However, smoking G-EOP patients typically failed to respond to *A. actinomycetemcomitans*, whereas smoking LJP patients responded normally. In short, G-EOP patients smoke very frequently, and it seems that smoking makes an enormous difference in the G-EOP response to *A. actinomycetemcomitans*. Thus, a major reason why the anti-*A. actinomycetemcomitans* response of G-EOP is so low appears to relate to the consequences of smoking rather than to a fundamental difference in the ability to respond to oral pathogens.

The previous observation that white subjects, independent of their disease status, have lower serum IgG concentrations than do their black counterparts was confirmed and extended; such differences were apparent even after smoking was taken into consideration (Tables 3 and 5). Clearly, smoking has effects on both serum Ig levels and on periodontal status, but this is not true of all periodontal groups when race is taken into account. The factors that determine how smoking will affect periodontal status and Ig production are not understood, and we look forward to future developments with interest.

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