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「口腔保健と全身的な健康状態の関係について」

C．研究協力課題名：「高齢者における歯周組織の状態と血清 IgG サブクラス量」

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E．研究目的：

歯周病原細菌に対する体液性免疫応答の主体をなすのは免疫グロブリン G（IgG）である。IgG は構造の違いにより IgG1ーIgG4 の４つのサブクラスに分類されている。各サブクラスは標的となる抗原の違いによりその産生を調節されている。IgG1 と IgG3 はタンパク質抗原に対して、IgG2 は糖タンパクに対して特異的に産生されることが知られている。これまで、多くの研究者により血清総 IgG 量あるいは血清 IgG サブクラス量と歯周病の病態との関連性が報告されてきた。Wilton らの研究では、成人性歯周炎患者の血清 IgG2 量は健常者に比べ有意に高かったと報告されている。また、Lu らの研究では、黒人の若年性歯周炎患者は血清 IgG2 量が同年齢の対照者に比べ有意に高かったと報告されている。また、Gunsolley らの研究では、血清 IgG 量および血清 IgG サブクラス量は人種などの遺伝的素因や、喫煙などの環境因子により影響を受けていると報告されている。

そこで本研究では、末梢血から得られる情報により歯周病に対する感受性を予測可能かを探る目的で、日本人における血清総 IgG 量および血清 IgG サブクラス量と歯周病の病態との関連性を検討した。

F．材料と方法：

1998 年４月の時点で新潟市に住民票を有する 71 歳の高齢者、451 名（男性 239 名、

女性 212 名）を選択し、アンケートによる問診と口腔内診査を行った。口腔内診査として、残存歯数、歯周ポケット深さ（PPD)、アタッチメントロス量（PAL)、歯石付着の有無（CAL)、プロービング時出血（BOP）の有無、について全歯にわたり測定した。また、各被験者から静脈血を採取し血清を分離後-80℃にて冷凍保存した。

総 IgG 量は免疫比濁法により測定した。血清 IgG サブクラス量は ELISA 法により測定した。つまり、リン酸緩衝生理食塩水（PBS）で希釈した抗ヒト IgG サブクラス抗体により 96 穴マイクロタイタープレートをコーティングし、5%スキムミルク含有 PBS でブロッキングした。0.05%Tween 20 含有 PBS で洗浄後、連続希釈したヒト標準血清あるいは被験血清を加え反応させた。洗浄後、アルカリホスファターゼ標識ヤギ抗ヒト IgG 抗体を加え反応させた。その後、基質として *p*-ニトロフェニルホスフェートを加えて発色させ 405 nm における吸光度を測定した。標準曲線より各被験血清の IgG サブクラス量を算出した。

Porphyromonas gingivalis 381 株を培養し、遠心分離によって得られた培養上清から線毛を分離･精製した。96 穴マイクロタイタープレートに線毛をコーティングし、5%スキムミルク含有 PBS でブロッキングした。標準血清あるいは被験血清をプレート上で反応させ、洗浄後、抗ヒト IgG サブクラス抗体を反応させた。洗浄後、アルカリホスファターゼ標識ヤギ抗マウス IgG 抗体を反応させた。その後、*p*-ニトロフェニルホスフェートを加えて発色させ 405 nm における吸光度を測定した。標準曲線より各被験血清の線毛に対する抗体価を算出した。

血清コチニン量は Nicotine Metabolite Kit (DPC 社) により測定した。コチニン量が調製された標準コチニン 25 µl と被験血清 25 µl をそれぞれ ^{125}I でラベルしたコチニン 100 µl と反応させ、さらに Nicotine Metabolite Antiserum 100 µl を加え反応させた。その後、遠心分離し上清を除去後、ガンマーカウンターにて CPM を測定した。得られたスタンダードの CPM 値から標準曲線を描き各被験血清のコチニン量を算出した。

残存歯数に基づき被験者を 2 群にわけ、群間における各パラメーターの検定に Wilcoxon の検定を用いた。

G．結果および考察：

選択した被験者 451 名の残存歯数は平均 17.41 本であった。歯周臨床検査データが得られないため、まず、無歯顎の被験者を除外した。次に、アンケート結果から喫煙者を除外しさらに血清中コチニン量が 100 ng/ml 以上であった被験者を除外した 340

名についてその後の解析を行った。残存歯の分布状態から 340 名を残存歯の少ない群（19 本以下）と多い群（20 本以上）の 2 群に分けた。解析にあたって、PPD、PAL は 4 mm 以上の部位の割合を、BOP、CAL はそれぞれ認められた部位の割合を算出した。その結果、2 群間で統計学的に有意差が認められたのは PAL が 4 mm 以上の部位の割合（p=0.001）、血清 IgG1 量（p=0.035）、血清 IgG2 量（p=0.025）、血清 IgG3 量（p=0.011）、血清 IgG4 量（p=0.022）であった。PPD 4 mm 以上の部位の割合、BOP（+）、CAL（+）の部位の割合、血清総 IgG 量、抗線毛 IgG および IgG サブクラス抗体価、血清コチニン量には有意差は認められなかった。

歯周病への感受性を示す指標については統一した見解が得られていないのが現状であるが、本研究ではその指標として残存歯数を選択した。しかし、残存歯の少ない群では PAL 4 mm 以上の部位の割合が有意に高かったことから残存歯の少ない群ではより歯周組織の破壊が進んでいることが明らかになった。また、残存歯の少ない群では血清 IgG1 量が有意に高く血清 IgG2〜4 量は有意に低かった。新潟市在住の高齢者を対象とした Sugita らの研究では、有意差は認められなかったが歯周病感受性が高い群では血清 IgG1 量および IgG3 量が高いという同様な所見が報告されている。残存歯の少ない群、つまり歯周組織の破壊が進んでいる群で血清 IgG1 量が高いという結果は，血清 IgG1 が歯周組織の破壊に関連してることを示唆している。一方，残存歯数の多い群、つまり歯周組織の破壊が進んでいない群では血清 IgG2〜4 量が高いという結果は、血清 IgG2〜4 が歯周組織の破壊に対する生体防御反応に関連していることを示唆している。*P. gingivalis* 線毛に対する IgG および IgG サブクラス抗体価については 2 群間には有意差を見出すことができなかった。Amano らは *P. gingivalis* 381 株の fim A 遺伝子型は、歯周炎患者から多く検出されるタイプ II ではなく健常者から多く検出されるタイプ I であったと報告している。fim A 遺伝子型がタイプ II である線毛を抗原として用いれば，2 群間に有意差が認められるかもしれない。

H．結論：

本研究では、末梢血から得られる情報により歯周病に対する感受性を予測可能かを探る目的で、高齢者における血清総 IgG 量および血清 IgG サブクラス量と歯周病の病態との関連性を検討した。その結果，末梢血中の血清 IgG サブクラス量を測定することにより、歯周病に対する感受性を知り得る可能性が明らかになった。

I．研究発表論文：

投稿原稿

Title

The association between serum IgG subclass levels and the periodontal status in an elderly Japanese population

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Running title: IgG subclass levels in elderly Japanese

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Abstract

A recent study suggested that genetic and environmental factors, such as race and smoking, can interact to influence levels of serum IgG subclasses. Japanese population living in the same area may be an appropriate subject to evaluate the association between IgG subclass levels and periodontal disease, since they have similar racial and environmental factors. The present cross-sectional study was aimed to evaluate the association between serum IgG subclass levels and the periodontal status in an elderly population. Elderly subjects (n=451, 71-years old) living in Niigata City, Japan were participated in the present study. Clinical evaluations including probing pocket depth, probing attachment level (PAL), bleeding on probing, and calculus, and serological evaluations including serum IgG and IgG subclass levels, anti-*Porphyromonas gingivalis* fimbriae IgG and IgG subclass titers and serum cotinine levels were performed. Smokers and edentulous subjects were first excluded from the 451 subjects. Dentulous nonsmokers were then classified into two groups based on the number of teeth: The periodontitis-susceptible subjects who had less than 20 teeth and the periodontitis-resistant subjects who had 20 or more teeth. Percentage of sites with $\geq$ 4 mm of PAL and serum IgG1 levels was significantly higher in the susceptible subjects, moreover, there was a significant negative correlation between the number of teeth and IgG1 subclass levels. These data indicate that higher levels of serum IgG1 is associated with smaller number of teeth in Japanese elderly populations. Longitudinal study is needed to clarify the reason for the higher serum IgG levels in the periodontitis-susceptible subjects.

Introduction

Number of teeth is decreased in elderly population. A recent survey of dental disease in Japan showed that average numbers of teeth are 27.52, 24.21, 21.98 and 14.33 at the age of 40, 50, 60 and 70, respectively [1]. A periodontal status evaluated by Community Periodontal Index [2] in the survey suggested that decrease in the number of teeth is caused mainly by periodontal disease in the elderly population.

IgG subclass antibodies have different biological properties, such as complement activation and binding to Fc receptors on phagocytes [3]. Therefore, the various clinical courses in infectious disease may be a result of the particular biological properties of a certain IgG subclass. Wilton et al. showed that serum IgG2 levels of patients with adult periodontitis was significantly elevated compared with the controls [4]. Tew et al. focused on IgG subclass response of the patients with early-onset periodontitis in their series of studies [5-7]. They showed that serum IgG2 levels of patients with localized juvenile periodontitis was elevated compared with age- and race-matched controls, indicating that the high levels of serum IgG2 in LJP patients is helpful in localizing periodontal destruction. Gunsolly et al. demonstrated that genetic and environmental factors, such as race and smoking, can interact to influence levels of individual IgG subclasses [8]. Therefore, it may be necessary to exclude these factors for evaluating the association between serum IgG subclass levels and periodontal status. Japanese population living in the same area has similar racial backgrounds, and may be an appropriate subject for the evaluation. However, the report on the association between IgG subclass levels and periodontal status in Japanese population is very limited. The purpose of the present cross-sectional study was to evaluate the association between serum IgG subclass levels and the periodontal status in a population of Japanese elderly subjects.

Material and methods

Subjects and clinical evaluations

An oral health survey of the elderly population was conducted in 1998 by the Ministry of Health and Welfare of Japan. Questionnaires were sent to all 6629 residents age 70 or 80 years old in Niigata City, Japan. Among these, 599 persons 70 years of age agreed to undergo the medical and dental examinations, with signed informed consent to the protocol, which was reviewed and approved by the Ethics Committee of the Faculty of Dentistry, Niigata University [9]. In the present study, 451 Japanese subjects (239 in males, 212 in female) of the 599 persons, who visited next year for participating in 2-year longitudinal study, aged 71 years old, were selected (screened population). Sera were obtained from all the subjects by venipuncture at the time of clinical evaluations, and stored at -80°C until the day before the assay.

Four dentists performed clinical evaluations on the following items: (i) number of teeth, (ii) probing pocket depth (PPD), (iii) probing attachment level (PAL), (iv) bleeding on probing (BOP), (v) calculus (CAL). PPD, PAL, BOP and CAL were assessed with a Williams probe at six sites per tooth.

Determination of serum IgG and IgG subclass levels

IgG levels were determined by immunoprecipitation assay at BML Inc., Tokyo, Japan. IgG subclass levels were determined by enzyme-linked immunosorbent assay (ELISA). First, 96-well microtiter plates (Nunc-Immuno® Plate II, Nalge Nunc International Co., Rochester, NY) were coated with one of anti-human IgG subclass monoclonal antibodies (Calbiochem-Novabiochem Co., San Diego, Calif.), HP6069A (anti-G1), HP6002 (anti-G2), HP6047A (anti-G3) and HP6023 (anti-G4), overnight at 4°C. After blocking unoccupied sites with phosphate-buffered saline (PBS) containing 5% non-fat dry milk, serial two-fold dilution of a human standard serum (1:500 to 1:512000; NOR-01, batch 4674, Nordic Immunology Laboratories, Netherlands) or diluted serum samples (IgG1; 1:4000, IgG2, 4; 1:16000, IgG3; 1:64000) were added to the well and incubated overnight at 4°C. A 1:1000 dilution of alkaline phosphatase-conjugated goat anti-human IgG antibody (Sigma Chemical Co., St. Louis, Mo.) was added, and plates were incubated for 4 h at room temperature.

Plates were washed three times with PBS containing 0.05% Tween 20 between each step. The color was developed by using *p*-nitrophenylphosphate as the substrate and optical density (OD) at 405 nm was measured. The data were fitted to a graph of the common logarithm of serum dilutions versus OD by 4-parameter logistic regression analysis. IgG subclass levels in serum samples were calculated from the standard curve.

Randomly selected twenty serum samples were measured for IgG subclass levels by a single radial immunodiffusion assay (Human IgG subclass SRID kit; The Binding Site Ltd., UK). IgG subclass levels measured by ELISA are significantly correlated with the levels measured by SRID with correlation coefficients of 0.799 (IgG1), 0.853 (IgG2), 0.649 (IgG3) and 0.625 (IgG4).

Determination of anti-fimbriae IgG and IgG subclass titers

Porphyromonas gingivalis 381 were anaerobically cultured in GAM broth (Nisui Pharmaceutical, Co., Ltd, Tokyo, Japan) at 37°C. Fimbriae was prepared from culture supernatant and purified by the method of Yoshimura et al. to check its purity on SDS-PAGE gels [10].

Microtiter plates (Nalge Nunc Intl.) were coated with purified fimbriae (0.2 μg/ml) overnight at 4°C. After blocking unoccupied sites, serial two-fold dilution of pooled high-titer human sera (1:64 to 1:8192) or a 1:500 dilution of serum samples were added to the well and incubated overnight at 4°C. A 1:4000 dilution of one of the anti-human IgG subclass monoclonal antibodies was added, then a 1:1000 dilution of alkaline phosphatase-conjugated goat anti-mouse IgG antibody (Sigma) was added, and plates were incubated for 4 h at room temperature.

Plates were washed three times between each step. The color was developed as described the above. The data were fitted to a graph of the common logarithm of serum dilutions versus OD by 4-parameter logistic regression analysis. Anti-fimbriae IgG subclass titers were calculated from the standard curve of pooled high-titer human sera.

Cotinine assay

Exposure to tobacco products was assessed by measuring serum cotinine, a stable metabolite of nicotine, by liquid-phase radioimmunoassay (Double antibody nicotine metabolite kit; Diagnostic Products Co., CA). First, 25 μl of serum sample or standard, 100 μl of ^{125}I-labeled cotinine and 100 μl of nicotine metabolite antiserum were added to polypropylene tubes, and the tube was incubated for 30 min at room temperature. After adding cold precipitating solution, the tube was centrifuged for 15 min at 3000 X *g*. Supernatant was removed, and the precipitate was measured with Gamma counter. Serum cotinine levels were calculated from the standard curve.

Statistical analysis

Percentages of sites with PPD ≥ 4 mm, PAL ≥ 4 mm, BOP (+) or CAL (+) were calculated for each subject. Results were expressed as mean ± SD. A software program (JMP4, SAS Institute Inc., Cary, NC) was used for statistical analysis. The differences in clinical and serological variables were examined using Mann-Whitney U test. In addition, the differences in ratio of male to female were examined using Chi-square test. The correlation between number of teeth and IgG subclass levels were examined using Spearman's rank-order correlation test.

Results

Smokers and edentulous subjects were excluded from 451 Japanese subjects (screened population) as shown in Table 1, since IgG subclass levels could be affected by smoking and no data on clinical variables were available for edentulous subjects. Smokers were excluded first based on the questionnaire, and then excluded on serum cotinine levels of ≥ 100 ng/ml. There was no significant difference in clinical and serological variables between screened population and dentulous nonsmoker except in the ratio of males to females. Significant decrease in number of males in dentulous nonsmoker mainly resulted from excluding a number of male smokers. Anti-fimbriae IgG3 titers could not be determined, since the titers of all serum samples were below the detecetable levels.

The distribution of number of teeth in based on the questionnaire, and then excluded on serum cotinine levels of ≥ 100 ng/ml was shown in Fig. 1. The distribution curve appeared to have two peaks at 10 and 25 of the number of teeth, respectively. The dentulous nonsmoker were then classified into two groups based on the number of teeth; the periodontitis-susceptible subjects who had less than 20 teeth and the periodontitis-resistant subjects who had 20 or more teeth.

Comparison of clinical and serological variables between the periodontitis-susceptible subjects and the periodontitis-resistant subjects was shown in Table 2. There was no significant difference in the ratio of male to female between the two groups of subjects. Percentage of sites with ≥ 4 mm of PAL was significantly higher in the susceptible subjects, while no significant difference was found for other clinical variables. Serum IgG levels were not significantly different between the two groups of subjects. However, IgG1 levels were significantly higher, and other IgG subclass levels were significantly lower in the susceptible subjects. There was no significant difference in the anti-fimbriae IgG or IgG subclass titers between the two groups of subjects. Serum cotinine levels was higher in the resistant subjects, however, the difference was not significant.

Correlations between the number of teeth and four IgG subclass levels were shown in Fig. 2. A negative correlation between the number of teeth and IgG1 subclass levels (correlation coefficient=-0.13, p=0.02) was found, while correlations between the number of teeth and the other IgG subclasses were not significant.

Discussion

Smoking is an important risk factor for both early-onset periodontitis and adult periodontitis [11]. Smoking has been shown to decrease serum IgG2 levels and alter PMN function [12]. Only questionnaires have been used in most epidemiological studies to examine smoking status. Some true smokers and exsmokers were misclassified as nonsmokers with the questionnaires only, and the misclassification may bias estimates of morbidity associated with smoking [13]. Suadicani et. al used serum cotinine as an objective marker of use of tobacco to examine characteristics of potentially misclassified smokers with respect to mortality, morbidity, and risk factors [14]. A serum concentration of 100 ng/ml was regarded as a relevant threshold for active smoking. In the present study, both questionnaires and the same threshold levels of serum cotinine were used to exclude active smokers. There was no significant difference in serum cotinine levels between the susceptible subjects and the resistant subjects in dentulous nonsmokers (Table 2). It might be necessary to consider possible effects of passive smoking on periodontal status, since serum cotinine is also used to determine the exposure of nonsmokers to tobacco. However, we did not consider the effects in the present study since threshold levels of cotinine for passive smoking were varied among the studies.

Susceptibility to periodontitis have been defined in various ways. Simply, subjects with gingivitis and subjects with chronic adult or rapidly progressive periodontitis of similar ages were defined as periodontitis-resistant subjects and periodontitis-susceptible subjecets, respectively [15-17]. Interleukin-1 genotype was also used to determine susceptibility to periodontitis [18]. Sugita et al. investigated a 70-years-old Japanese population to determine whether the FcγRIIIb polymorphism was associated with resistance to periodontitis [9]. They selected nonsmokers with more than 20 teeth to define the periodontitis-resistant group as having $\leq 5\%$ of sites with PAL ≥ 4 mm, and the periodontitis-susceptible group as having $\geq 20\%$ sites with PAL ≥ 4 mm. In the present study, smokers and edentulous subjects were excluded from screened population to select dentulous nonsmokers for further analysis. The distribution curve of number of teeth in dentulous nonsmokers appeared to have two peaks (Fig. 1). We assumed that subjects with smaller number of teeth are more susceptible to periodontitis in the present elderly population of the same age, although causes of tooth loss were not clear. On the assumption, dentulous nonsmokers were classified into two groups based on the number of teeth: The periodontitis-susceptible subjects (number of teeth < 20) and the periodontitis-resistant subjects (number of teeth ≥ 20). Certainly, percentages of sites with PAL ≥ 4 mm were significantly higher in periodontitis-susceptible subjects indicating that periodontal breakdown

was more severe in the periodontitis-susceptible subjects (Table 2). These results were not changed if we reclasssified the two groups of subjects based on the therethold number of teeth, 17, 18 and 19 teeth.

Mean levels of serum IgG1, G2, G3 and G4 in human are 9, 3, 1 and 0.5 mg/ml, respectively. In the present study, the mean levels of IgG3 were lower than IgG4. Wilton et al. also measured levels of serum IgG subclasses by ELISA to show the similar results [4]. We compared four IgG subclass levels of randomly selected sera measured by ELISA with those by SRID. The IgG3, 4 levels by SRID decreased while measured by ELISA, and the reduction was higher in IgG3 levels. Therefore, the contradiction of IgG3 and IgG4 levels may be due to the difference of ELISA and SRID.

Serum IgG levels were not significantly different between the periodontitis-susceptible subjects and the periodontitis-resistant subjects (Table 2). However, there were significant differences in serum IgG subclass levels: IgG1 levels were significantly higher and the other IgG subclass levels were significantly lower in the periodontitis-susceptible subjects. Percentages of IgG subclass levels in IgG levels were also calculated for each subjects. Ratios of the average percentage of the susceptible subjects to that of the resistant subjects were 58.28/53.50 for IgG1, 35.43/39.65 for IgG2, 2.96/3.50 for IgG3 and 3.73/4.53 for IgG4. There were significant differences between the percentages for all the IgG subclass. These data indicate that higher levels of IgG1 subclass in the periodontitis-susceptible subjects are not due to higher levels of IgG levels but result from higher percentages of IgG1 in IgG levels, and that higher levels of other IgG subclass the periodontitis-resistant subjects result from higher percentages of other IgG subclass in IgG levels.

A large number of plasma cells is known to infiltrate and produce IgG antibodies in periodontal tissue involved with periodontitis [19, 20]. However, it is not clear whether IgG antibodies locally or systemically produced play protective or destructive roles in periodontal tissue breakdown. In the present study, there was a negative correlation between the number of teeth and IgG1 levels (Fig. 2), indicating that subjects with smaller number of teeth had higher IgG1 levels, or subjects with higher IgG1 levels lost larger number of teeth. Total amount of dental plaque may decrease in acoording to decrease in number of teeth. If dental plaque elavates serum IgG1 production in host, decrease in the amount of plaque would leads to decrease in serum IgG1 levels. Therefore, subjects with higher IgG1 levels may lost larger number of teeth.

We have to carefully consider vaccination in periodontal treatment if a vaccine against proteinous antigens including *P.*

gingivalis fimbriae, *Actinobacillus actinomycetemcomitans* leukotoxin is used. The vaccine would elevated serum IgG1 levels to lead periodontal tissue breakdown.

P. gingivalis has many virulence factors including gingipain and fimbriae. We chosen its fimbriae as a specific antigen to measure specific IgG response to *P. gingivalis*, since this organism is considered to be involved in adult periodontitis. In the present study, the assay for anti-fimbriae titers did not work only for IgG3 subclass.

We have carefully checked antigen-antibody bindings in each step of the assay to conclude that anti-fimbriae IgG3 antibody was not detectable in sera from the subjects. Ogawa et al. reported that the major response to fimbriae from *P. gingivalis* 381 was IgG3 followed by IgG1, IgG2 and IgG4 in patients with periodontal disease and the control subjects [21]. Possible reason for this contradiction may result from the difference of the assay. However, a recent study showed that the major response to whole cells of *P. gingivalis* 381 was IgG1 followed by IgG2 and IgG4, with minimal levels of IgG3 [22].

There was no significant difference in the anti-fimbriae IgG or IgG1, 2, 4 titers between the susceptible- and the resistant- subjects in the present study,. Amano et al. investigated that the relationship between the prevalence of fimA genotypes of *P. gingivalis* and periodontal health status in adults, showing that the most prevalent fimA types were type I in healthy adults (76.8%), and type II in periodontitis patients (66.1%), respectively [23]. *P. gingivalis* 381 which fimbriae we used in the present study was classified into type I fimA genotype. We may detect significant difference in IgG and IgG subclass response to fimbriae prepared from type II fimA genotype of *P. gingivalis*.

In summary, our results suggested that higher levels of serum IgG1 is associated with smaller number of teeth in Japanese eldery populations. Many local and systemic factors other than periodontal status could affect serum IgG subclass levels. Longitudinal study is needed to clarify the reason for the higher serum IgG levels in the periodontitis-susceptible sub jects.

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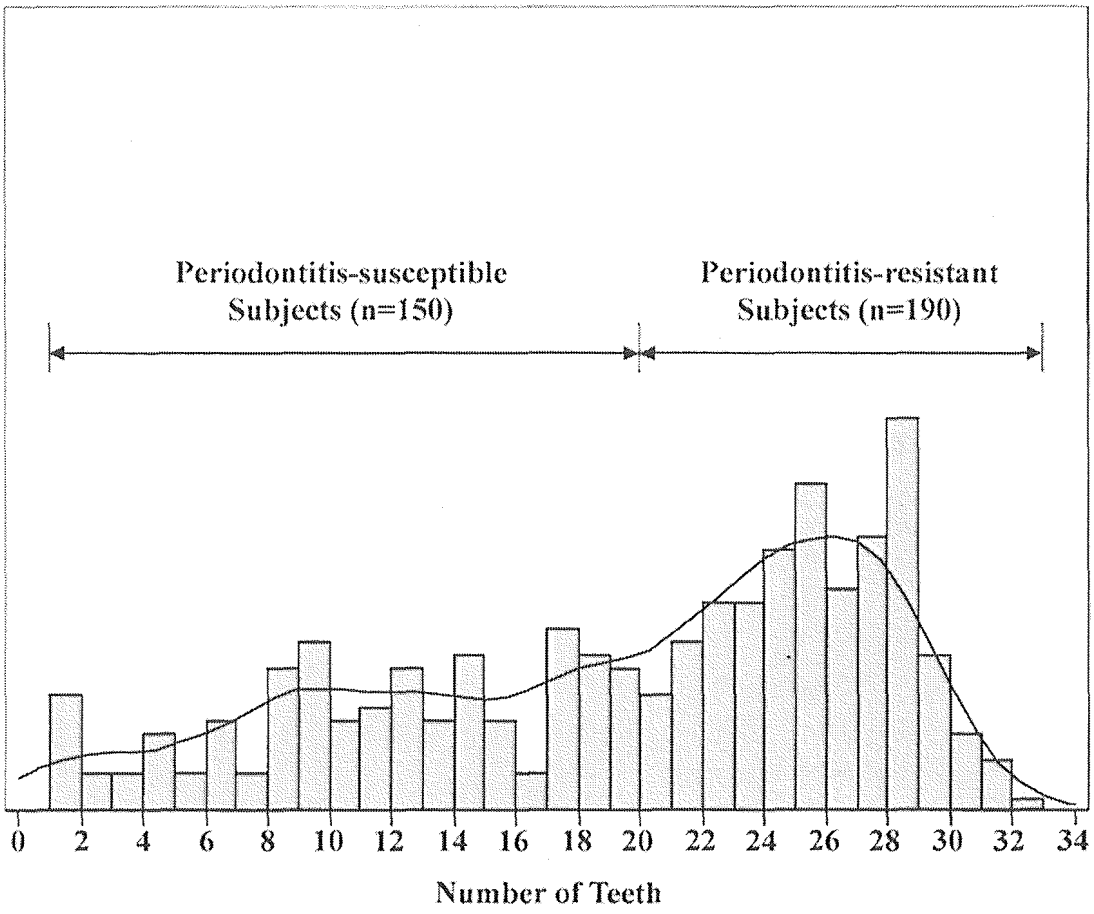
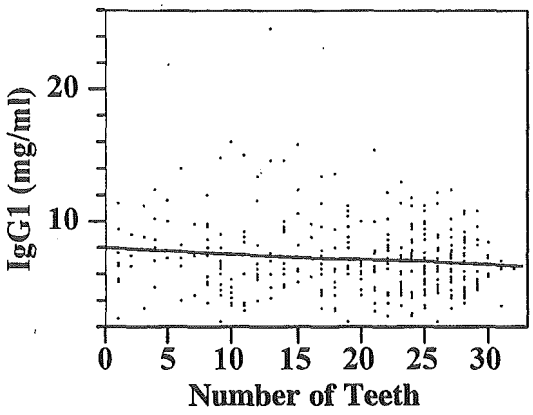
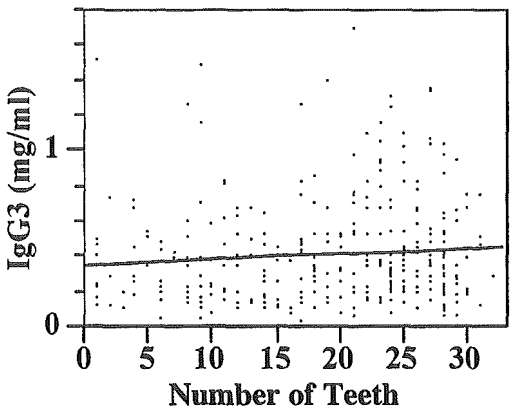
Figure legends

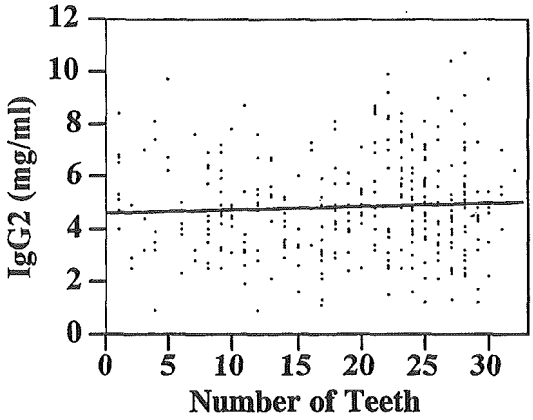
Fig. 1. Histogram of the number of teeth in dentulous nonsmokers
A smooth curve to the histogram was created using nonparametric density estimation with the kernel standard of 1.49. Dentulous nonsmokers were classified into two groups based on the number of teeth: The periodontitis-susceptible subjects who had less than 20 teeth and the periodontitis-resistant subjects who had 20 or more teeth.

Fig. 2. Correlations between the number of teeth and four IgG subclass levels
Correlation coefficient between the number of teeth and IgG subclass levels were −0.13 (p=0.02) for IgG1, 0.05 (p=0.39) for IgG2, 0.09 (p=0.10) for IgG3, and 0.09 (p=0.10) for IgG4.


Periodontitis-susceptible
Subjects (n=150)
Periodontitis-resistant
Subjects (n=190)
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34
Number of Teeth


IgG1 (mg/ml)
20
10
0 5 10 15 20 25 30
Number of Teeth


IgG3 (mg/ml)
1
0
0 5 10 15 20 25 30
Number of Teeth


IgG2 (mg/ml)
12
10
8
6
4
2
0
0 5 10 15 20 25 30
Number of Teeth

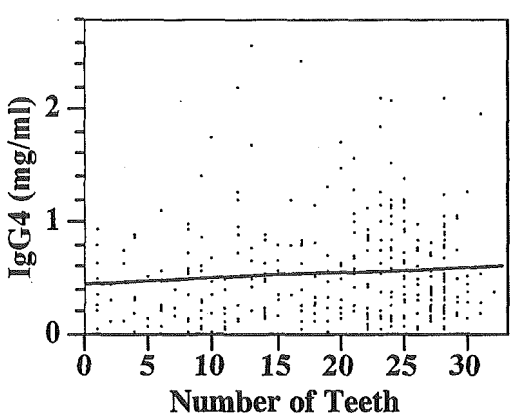

IgG4 (mg/ml)
2
1
0
0 5 10 15 20 25 30
Number of Teeth

Table 1. Clinical and serological variables in screened population and dentulous nonsmoker

Variables	Screened population (n=451)	Dentulous nonsmoker (n=340)	*p* value†
Male/Female	239/212	151/189	0.02
Smoker (%)	20.8	0	ND
Clinical Variables			
Number of teeth	17.41±9.31	19.14±8.10	ND
Percentage of sites (%)			
PPD ≥ 4 mm	10.66±13.07	9.66±12.55	0.23
PAL ≥ 4 mm	35.28±27.81	32.73±26.65	0.24
BOP (+)	7.14±8.52	7.40±8.78	0.70
CAL (+)	3.69±5.81	3.44±5.56	0.66
Serological Variables			
Serum IgG (mg/ml)	12.93±2.74	13.04±2.68	0.97
IgG1 subclass	7.19±2.90	7.16±2.79	0.85
IgG2 subclass	4.79±2.17	4.83±1.88	0.45
IgG3 subclass	0.43±0.32	0.41±0.29	0.45
IgG4 subclass	0.54±0.45	0.54±0.44	0.95
Serum anti-fimbriae IgG (EU)	1218±966	1296±1023	0.25
IgG1 subclass	994±1356	1081±1437	0.28
IgG2 subclass	1705±1302	1760±1359	0.60
IgG4 subclass	488±443	501±453	0.63
Serum cotinine (ng/ml)	45.07±99.91	5.20±8.36	ND

†The differences in clinical and serological variables were examined using Mann-Whitney U test. while the differences in ratio of male to female were examined using Chi-square test. ND: not determined. Serum anti-fimbriae IgG3 titers were not detected.

Table 2. Clinical and serological variables in periodontitis-susceptible subject and the periodntitis-resistant subject

Variables	Periodontitis-susceptible Subject (n=150)	Periodontitis-resistant Subject (n=190)	*p* value†
Male/Female	64/86	87/103	0.65
Clinical Variables			
Number of teeth	11.31±5.33	25.32±2.86	ND
Percentage of sites (%)			
PPD ≥ 4 mm	11.16±14.41	8.47±10.75	0.56
PAL ≥ 4 mm	42.82±29.35	25.10±21.50	< 0.01
BOP (+)	8.64±10.41	6.42±7.13	0.21
CAL (+)	3.72±6.58	3.22±4.60	0.07
Serological Variables			
Serum IgG (mg/ml)	13.12±2.81	12.98±2.58	0.87
IgG1 subclass	7.64±3.29	6.78±2.27	0.04
IgG2 subclass	4.55±1.70	5.04±1.99	0.03
IgG3 subclass	0.39±0.28	0.44±0.30	0.01
IgG4 subclass	0.49±0.44	0.58±0.44	0.02
Serum anti-fimbriae IgG (EU)	1249±976	1332±1059	0.48
IgG1 subclass	1048±1421	1107±1452	0.65
IgG2 subclass	1792±1446	1734±1291	0.68
IgG4 subclass	471±416	523±478	0.40
Serum cotinine (ng/ml)	4.45±8.36	5.78±8.33	0.06

†The differences in clinical and serological variables were examined using Mann-Whitney U test. while the differences in ratio of male to female were examined using Chi-square test. ND: not determined. Serum anti-fimbriae IgG3 titers were not detected.