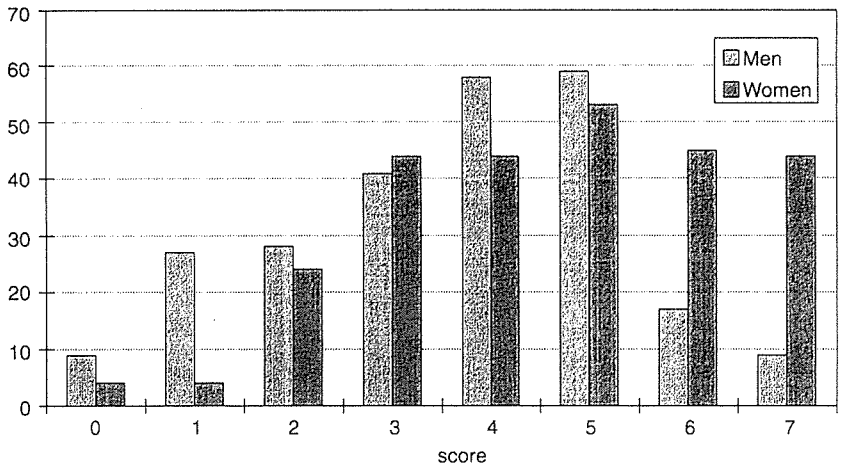

Figure 1 Number of subjects in subgroups of dental health knowledge score.

significant in women. Women with full-time employments had a significantly higher rate of using dental floss than unemployed women. Age classification was a contributing factor to both using dental floss in men and dental check-ups in women. In men, no variable was significantly associated with toothbrushing frequency and dental check-ups.

Discussion

The state of family composition of the subjects was roughly equal to that of adults aged 20-29 in Chiba City (Men: live alone 27.9%, live with family 72.1%, Women: live alone 14.0%, live with family 86.0%). Similarly, the rate of full-time workers among the subjects was roughly equal to that of adults aged 20-29 in Chiba City (Men: 71.1%, Women: 58.9%). From these aspects, the subjects in this study are thought to be representative.

Subjects of this study were young, so few were managers or professionals. We hypothesized that being employed or unemployed and, if employed, whether full-time or part-time, would be related to oral health behaviour. Therefore, as a socio-demographic variable, we used employment status (full-time, part-time, unemployed). A similar classification was used in other study.[9] Recently, the decline of health behaviour of young people who live alone is commonly pointed out in Japan. Therefore, we hypothesized that a difference would be seen between those who live alone or who live with family. For these reasons, employment status and family composition are thought to be proper as socio-demographic factors in Japanese young adults.

Women exhibited higher dental knowledge scores than men which is consistent with other reports.[15,16] Also, women exhibited better oral health behaviour than men. This finding coincides with findings of other reports.[7,8,11] It is thought that improvement of oral health knowledge and of oral health behaviour is required for men aged 20-29. Most of these subjects are salaried worker in Japan. Health education and instruction in health promotion have scarcely been provided to workers in Japan. Health promotion for workers may increase awareness of health, particularly in men.

In men, a significant relationship was seen only between using dental floss and age. However, for women, each oral health item had associated factors. Dental health knowledge was associated with using dental floss in women. Dental floss is easily acquired on the market in Japan. It is suggested that women are more ready to exhibit good health behaviour than men by acquiring health knowledge, if such behaviour is easy to carry out. In addition to dental health knowledge, employment status was significantly correlated with using dental floss in women. The social activity of being employed full-time may influence oral health behaviour in women.

Table 4 State of oral health behaviour.

	Men	Women	*p*
Toothbrushing frequency (per day)			
Two or more	154 (63.4)	249 (88.3)	0.001
Less than 2	89 (36.6)	33 (11.7)	
Dental floss			
Use	74 (30.7)	108 (38.3)	0.042
Not use	167 (69.3)	174 (61.7)	
Dental check-up (per year)			
One or more	11 (4.5)	48 (17.2)	0.001
Less than 1	231 (95.5)	231 (82.8)	

Note: missing data were not included in calculation.

Table 5 Odds ratios from multiple logistic regression analyses of oral health behaviour.

	Odds ratio (95% CI)		
	Toothbrushing frequency	Dental floss	Dental check-ups
Men			
Age group			
20-24	1.0...	1.0...	1.0...
25-29	1.06 (0.60-1.88)	2.00 (1.09-3.68)*	2.32 (0.55-9.71)
Employment status			
Unemployed	1.0...	1.0...	1.0...
Part-time	0.35 (0.03-4.12)	[a]	[a]
Full-time	1.25 (0.66-2.37)	1.22 (0.59-2.52)	2.51 (0.29-22.10)
Family composition			
Live alone	1.0...	1.0...	1.0...
Live with family	1.06 (0.59-1.90)	0.75 (0.41-1.37)	1.43 (0.36-5.70)
Dental health knowledge			
Lower	1.0...	1.0...	1.0...
Higher	1.37 (0.80-2.34)	1.33 (0.75-2.37)	1.88 (0.48-7.36)
Women			
Age group			
20-24	1.0...	1.0...	1.0...
25-29	0.69 (0.32-1.50)	1.04 (0.63-1.71)	2.16 (1.11-4.21)*
Employment status			
Unemployed	1.0...	1.0...	1.0...
Part-time	0.58 (0.17-2.04)	1.01 (0.38-2.66)*	0.22 (0.03-1.91)
Full-time	1.54 (0.64-3.74)	1.93 (1.06-3.51)	2.15 (0.93-5.01)
Family composition			
Live alone	1.0...	1.0...	1.0...
Live with family	2.71 (1.10-6.68)*	1.32 (0.63-1.71)	0.78 (0.35-1.74)
Dental health knowledge			
Lower	1.0...	1.0...	1.0...
Higher	1.48 (0.67-3.27)	1.87 (1.08-3.24)*	1.93 (0.91-4.07)

CI = confidence interval, $p < 0.05$

[a] OR and 95% CI cannot be calculated for dental floss and dental check-up because no part-time worker had good oral health behaviour in these categories.

Women aged 25-29 showed a significantly higher rate of having dental check-ups than women aged 20-24. Woolfolk et al.[17] reported that factors associated with dental health check-up frequency were gender, income level, having a usual place for dental care and anxiety about receiving dental care. In the present study, age was shown to be associated with having dental check-ups. In Japan, the rate of occurrence of periodontitis increases at about the age of 30. For oral health promotion, an increase in the number of persons who are concerned with professional care in the 25-29 age group is desirable.

Toothbrushing frequency was significantly associated with family composition in women. One study reports the relationship between family composition and health behaviour in young adults.[18] It is thought that living alone may affect the frequency of toothbrushing in young women. These findings suggest that the oral health behaviour of women is more readily influenced by various factors than that of men. Women were reported to have lower self-assessment of oral health,[15,16,19] greater dental anxiety,[20-23] and better dental attendance.[13,24] Women, harbouring these characteristics, may be ready to begin good oral health behaviour as they age or acquire dental health knowledge. Therefore, it is expected that the effect of health promotion is greater in young women than in young men. We previously reported that the relationship between caries treatment and smoking was significant in young women but in young men.[25] Health behaviour of men is more elusive than that of women. However, men may have some determinants other than the factors used in this study. Improvement of oral health behaviour of young men needs some

motivation. Motivation to improve oral health behaviour in young men is important. It is necessary to investigate factors other than those examined here are associated with oral health behaviour in young men.

In this study, sexual differences were seen in factors associated with oral health behaviour. Factors associated with oral health behaviour may differ in adults in other age groups. Similar analysis on that point by gender will yield useful findings for oral health promotion.

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Establishment of an Animal Model Using Recombinant NOD.*B10.D2* Mice To Study Initial Adhesion of Oral Streptococci

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An oral biofilm is a community of surface-attached microorganisms that coats the oral cavity, including the teeth, and provides a protective reservoir for oral microbial pathogens, which are the primary cause of persistent and chronic infectious diseases in patients with dry mouth or Sjögren's syndrome (SS). The purpose of this study was to establish an animal model for studying the initial adhesion of oral streptococci that cause biofilm formation in patients with dry mouth and SS in an attempt to decrease the influence of cariogenic organisms and their substrates. In nonobese diabetogenic (NOD) mice that spontaneously develop insulin-dependent diabetes mellitus (IDDM) and SS, we replaced major histocompatibility complex (MHC) class II (A^{g7} E^{g7}) and class I D^b with MHC class II (A^d E^d) and class I D^d from nondiabetic B10.D2 mice to produce an animal model that inhibited IDDM without affecting SS. The adhesion of oral streptococci, including *Streptococcus mutans*, onto tooth surfaces was then investigated and quantified in homologous recombinant N5 (NOD.*B10.D2*) and N9 (NOD.*B10.D2*) mice. We found that a higher number of oral streptococci adhered to the tooth surfaces of N5 (NOD.*B10.D2*) and N9 (NOD.*B10.D2*) mice than to those of the control C57BL/6 and B10.D2 mice. On the basis of our observation, we concluded that these mouse models might be useful as animal models of dry mouth and SS for in vivo biological studies of oral biofilm formation on the tooth surfaces.

Oral streptococci are present in large numbers in dental plaque, and several types interact with the enamel salivary pellicle to form a biofilm on tooth surfaces (9, 16, 17, 21, 29). Streptococci account for approximately 20% of the total number of salivary bacteria (24), with *Streptococcus salivarius* being the primary organism. Further, the densities of *Streptococcus mutans* and *Streptococcus sanguis* in saliva are more than 1×10^5 cells per ml. *S. mutans* is a pioneering organism that plays an important role in biofilm formation on tooth surfaces and is a primary causative agent of dental caries (9, 16, 21). The mechanical forces of salivary flow and tongue movement tend to dislodge and expel bacteria from tooth surfaces and the oral cavity (3, 5, 6), and their importance in controlling microbial colonization in the oral cavity has been well demonstrated in individuals with diabetes mellitus, Sjögren's syndrome (SS), and dry mouth, who suffer from a rapid overgrowth of biofilm and rampant caries, making them highly susceptible to oral infections (1–2, 6). Thus, attempts to investigate the initial adhesion by oral streptococci, including *S. mutans*, in mouse models are likely to aid in the understanding and prevention of oral infectious diseases caused by the components of oral biofilm.

Previous studies of *S. mutans* infections in the oral cavities of mice have been performed by feeding the animals diets containing sucrose in the presence of glucans (13, 15, 30, 43). Since the adherence of *S. mutans* to the tooth surface may depend on the balance between physical adherence and synthesis of in-

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TABLE 1. Linkage markers analyzed for homozygosity to NOD-derived *Idd* loci in NOD.*B10.D2* congenic mice

Idd locus/ chromosome	Linkage marker homozygous to NOD allele	Relative microsatellite size[a]
Idd1/17	D17Mit 198	B10.D2 > NOD
	D17Mit 195	B10.D2 > NOD
	D17Mit 194	B10.D2 = NOD
	D17Mit 173	B10.D2 = NOD
	D17Mit 145	B10.D2 = NOD
	D17Mit 82	B10.D2 > NOD
	D17Mit 34	B10.D2 > NOD
	D17Mit 28	B10.D2 > NOD
	D17Mit 59	B10.D2 = NOD
	D17Mit 62	B10.D2 = NOD
Idd2/9	D9Mit 25	B10.D2 = NOD
Idd3/3	D3Mit 95	B10.D2 < NOD
	D3Mit 103	B10.D2 = NOD
	D3Mit 206	B10.D2 = NOD
Idd4/11	D11Mit 115	B10.D2 < NOD
	D11Mit 320	B10.D2 < NOD
Idd5/1	D1Mit 46	B10.D2 < NOD
Idd6/6	D6Mit 15	B10.D2 > NOD
	D6Mit 339	B10.D2 < NOD
	D6Mit 52	B10.D2 > NOD
Idd7/7	D7Mit 20	B10.D2 > NOD
Idd8, Idd12/14	D14Mit 222	B10.D2 > NOD
	D14Mit 110	B10.D2 = NOD
Idd9, Idd11/14	D4Mit 59	B10.D2 < NOD
Idd10/3	D3Mit 103	B10.D2 = NOD
Idd13/2	D2Mit 257	B10.D2 < NOD
	D2Mit 17	B10.D2 = NOD
Idd14/13	D13Mit 61	B10.D2 < NOD
Idd15/15	D5Mit 48	B10.D2 > NOD

[a] Microsatellite markers with the indicated allelic size variants were typed in backcross mice used for the intercross.

soluble glucans in a natural environment, that infection method may be inappropriate for investigation of natural biofilm formation associated with streptococci, including *S. mutans* (18, 39).

The nonobese diabetogenic (NOD) mouse strain is currently the best available model for the study of insulin-dependent type 1 diabetes mellitus (IDDM) and SS (11, 31), both of which develop spontaneously and are characterized by lymphatic infiltration of the pancreas and salivary glands. Oral changes are prominent features of these diseases, which are manifested by dry mouth and hyposalivation (6, 7, 37). NOD mice are also used as an animal model for the study of oral infectious diseases associated with systemic diseases such as diabetes and SS or dry mouth.

The unique major histocompatibility complex (MHC) class II genes ($I\text{-}A^{g7}$, no expression of *I-E*) represent dominant susceptibility factors and mediate activated T cells during the development of diabetes in NOD mice (11, 22, 25, 36, 41, 42). In the NOD model of SS, histopathological analyses of the salivary glands in MHC-congenic strains of NOD mice have indicated that the $I\text{-}A^{g7}$ region is not required for lymphocytic infiltration (26, 31). Further, replacement of the NOD MHC

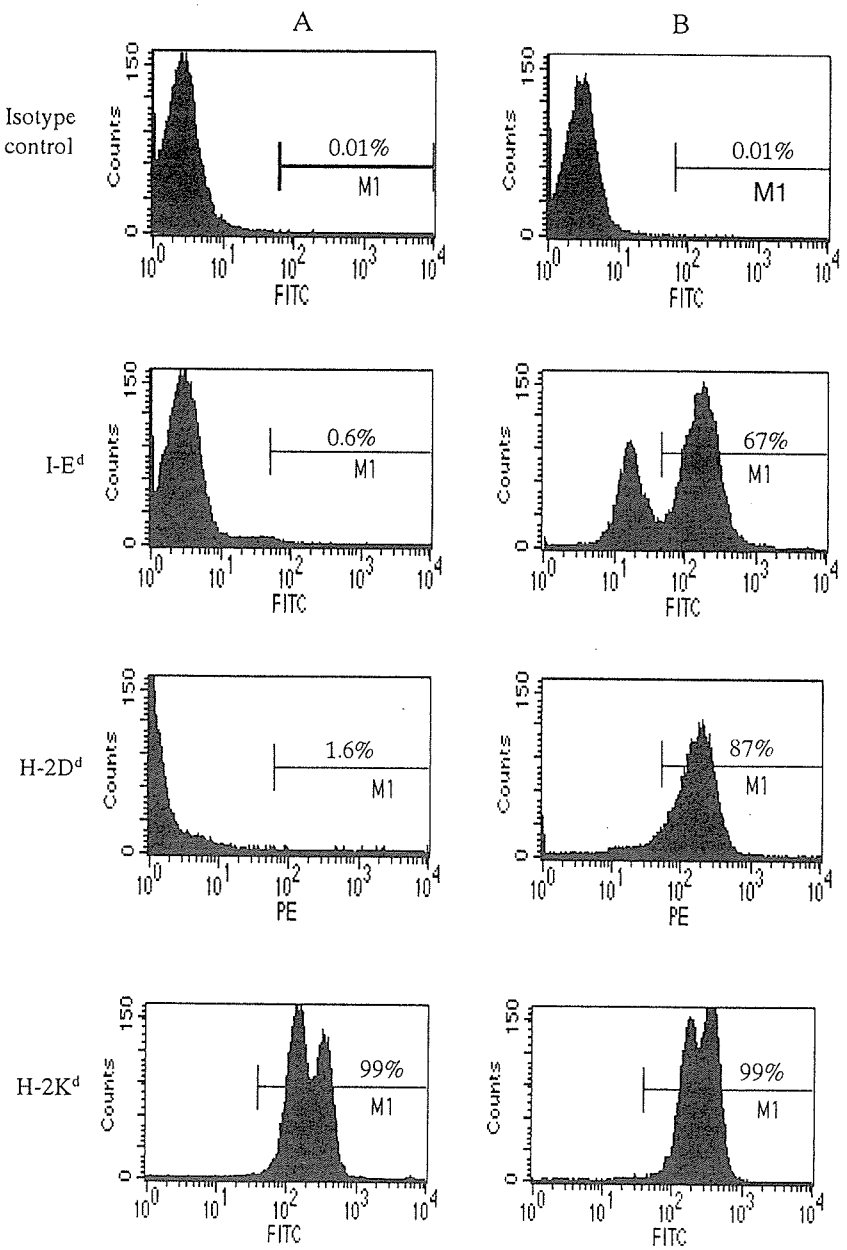


FIG. 1. Serological typing of lymphocytes. Spleen cells from NOD (A) and N5 (NOD.*B10.D2*) (B) female mice at 4 months of age were examined for the expression of MHC class I $H\text{-}2K^d$, MHC class II $I\text{-}E^d$, and $H\text{-}2D^d$ by using FACS analysis. NOD, MHC class II $I\text{-}E^d$, and $H\text{-}2D^d$ antigens were expressed in the NOD mice. Each histogram shows the percentage of total spleen cells from NOD and N5 (NOD.*B10.D2*) mice. Histograms are representative of the results of three independent experiments with 10 mice in each group, with similar results obtained in each experiment. FITC, fluorescein isothiocyanate; PE, phycoerythrin.

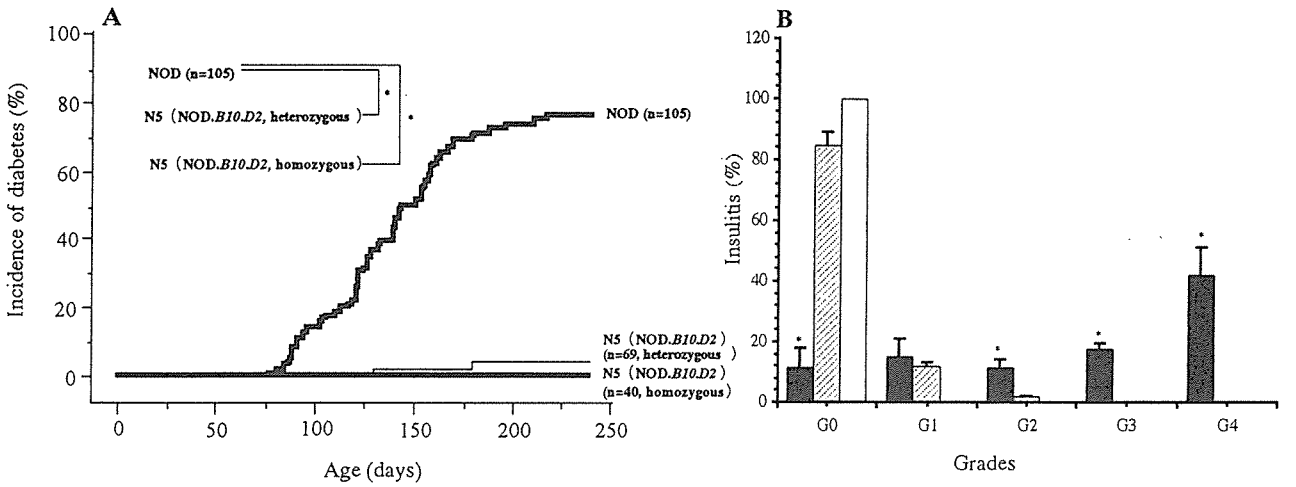


FIG. 2. Cumulative incidence of diabetes and insulitis in NOD and new N5 (NOD.*B10.D2*) mice. (A) Mice homozygous for B10.D2 MHC avoided diabetes in comparison with the heterozygous and NOD types. (B) Insulitis scores were calculated by using hematoxylin and eosin staining of pancreas sections from F_1 (□), N5 (▨), and NOD (■) female mice at 4 months of age. The degree of lymphocyte infiltration was graded as follows: G0, no infiltrating cells in the islets; G1, infiltrating cells adjacent to the islets; G2, infiltrating cells occupying less than 25% of the islets; G3, infiltrating cells occupying 25 to 50% of the islets; G4, infiltrating cells occupying more than 50% of the islets. For example, the percentage of G1 insulitis = (number of G1)/(G0 + G1 + G2 + G3 + G4 + G4) × 100. Results are expressed as the means ± standard deviations (SDs) of the results for 12 mice per strain. *, $P < 0.001$ for the results for NOD mice versus those for N5 and F_1 mice.

class I K^d region with another haplotype, MHC class I K^{wm7}, as well as replacement of the MHC class II A^{g7} E^{g7} and class I D^d regions with the corresponding region from the other MHC haplotype, has been shown to prevent diabetes (12). However, replacement with MHC class I *K* does not completely prevent development of insulitis. In another report, NOD mice pretreated nasally by using peptides restricted with MHC class I K^d showed a delayed onset of spontaneous IDDM, though insulitis could not be prevented by the induction of tolerance (23).

In the present study, we attempted to establish an animal model for oral infectious diseases such as dental caries by focusing on replacement of the MHC class II and class I *D* region but not the class I *K* region in nondiabetic NOD mice by outcrossing B10.D2 mice (K^d, $I\text{-}A^d$, and D^d) with NOD mice (K^d, $I\text{-}A^{g7}$, and D^b) because the MHC class I *K* region in B10.D2 mice is identical with that in NOD mice (12). The present backcrossed and intercrossed NOD mice with the MHC class II and MHC class I *D* region replaced with that from B10.D2 mice developed SS, however, not diabetes. We then attempted to determine whether these mice would be useful as animal models for a sucrose-free study of the initial adhesion of oral streptococci on tooth surfaces in humans.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The *Streptococcus* strains used in this study were *S. mutans* MT8148, *S. sanguis* ATCC 10556, *S. sobrinus* 6715, *S. salivarius* ATCC 9759, and *S. mitis* ATCC 6249. All bacteria were grown in an atmosphere of H_2 and CO_2 (GasPack; Becton Dickinson and Co., Franklin Lakes, N.J.) in brain heart infusion broth (Difco Laboratory, Detroit, Mich.) at 37°C overnight and were then harvested and washed twice with sterile phosphate-buffered saline (PBS).

Animals and assessment of diabetes and saliva. NOD/LtJ and B10.D2 mice were purchased from the Jackson Laboratory (Bar Harbor, Maine) and Japan SLC (Shizuoka, Japan), respectively, and maintained in accordance with the guidelines of the National Institute of Infectious Diseases. Clinical onset of diabetes in NOD mice was determined by the presence of glucose in urine and blood. Urine was tested weekly using Uristix reagent strips (Bayer Medical Ltd., Newbury, United Kingdom) and confirmed to be positive by blood glucose measurements. After being anesthetized, the mice were injected with a cocktail of isoproterenol (0.20 μg/100 gm of body weight) and pilocarpine (0. 05 μg/100 gm) (Sigma Chemical, St. Louis, Mo.) in PBS as a secretagogue. Following the intraperitoneal injection, saliva was collected from each mouse by using a micropipette for 15 min and was stored at −80°C.

Bacterial sampling and CFU counting. All oral streptococci were cultured in brain heart infusion broth overnight and were then washed twice with sterile PBS. Chlorhexidine (0.2%)-soaked sterile cotton swabs were used for disinfecting the oral cavities of the mice, including the lower incisor teeth, which were immediately washed with sterile PBS. Oral streptococci were introduced into the oral cavities at a final concentration of 7×10^9 CFU in 250 μl of PBS in all females at 4 or 8 months of age for 2.5 min, after which the mice were not provided food or drinking water. Following inoculation, samples were collected from the labial surfaces of the lower incisor teeth with a sterile cotton ball and then dipped into 2 ml of PBS. The samples in PBS were sonicated by ultrasonic dispersion (power output, 60 W) for 10 seconds and were then poured onto Mitis-Salivarius agar plates containing 0.02 M bacitracin (MSB) by using an EDDY JET spiral system (Gunze Sangyo, Inc., Tokyo, Japan). CFU were determined after 48 h of anaerobic incubation at 37°C.

Generation of backcross mice. To generate the backcross generation, NOD mice were mated with B10.D2 mice to produce (NOD × B10.D2)F_1 mice, and then heterozygous F_1 mice were mated with NOD mice to produce first-generation backcross (BC1) mice. BC1, BC2, BC3, and BC7 heterozygous mice were then mated with NOD mice to produce BC2, BC3, BC4, and BC8 mice, respectively, and BC4 and BC8 heterozygous mice were intercrossed to produce N5 and N9 MHC-recombinant NOD mice. After an outcross of the NOD strain to the B10.D2 strain, repetitive backcrossing with NOD mice was performed, with breeder selections based on genomic PCRs and/or simple sequence length polymorphism (SSLP) analysis with microsatellite markers (MapPairs; Research Genetics, Huntsville, Ala.), as shown in Table 1. The PCRs (25 μl) were done using a PTC-200 (MJ Research, Watertown, Mass.) for 40 cycles (94°C, 15 seconds; 58°C, 45 seconds; 72°C, 5 min, after an initial denaturation at 94°C for 3 min) and were then analyzed on a 3% agarose gel. An SSLP analysis of the *B10.D2* allele from the backcross generation identified the mice as homozygous for allelic variants characteristic of NOD mice with replacements of the MHC region with that from B10.D2 mice at all of the *Idd17* linkage markers (Table 1). Typing of these markers without *Idd17* confirmed the homozygous presence of the NOD-derived background genome at the identified *Idd* loci in the backcross generation (Table 1).

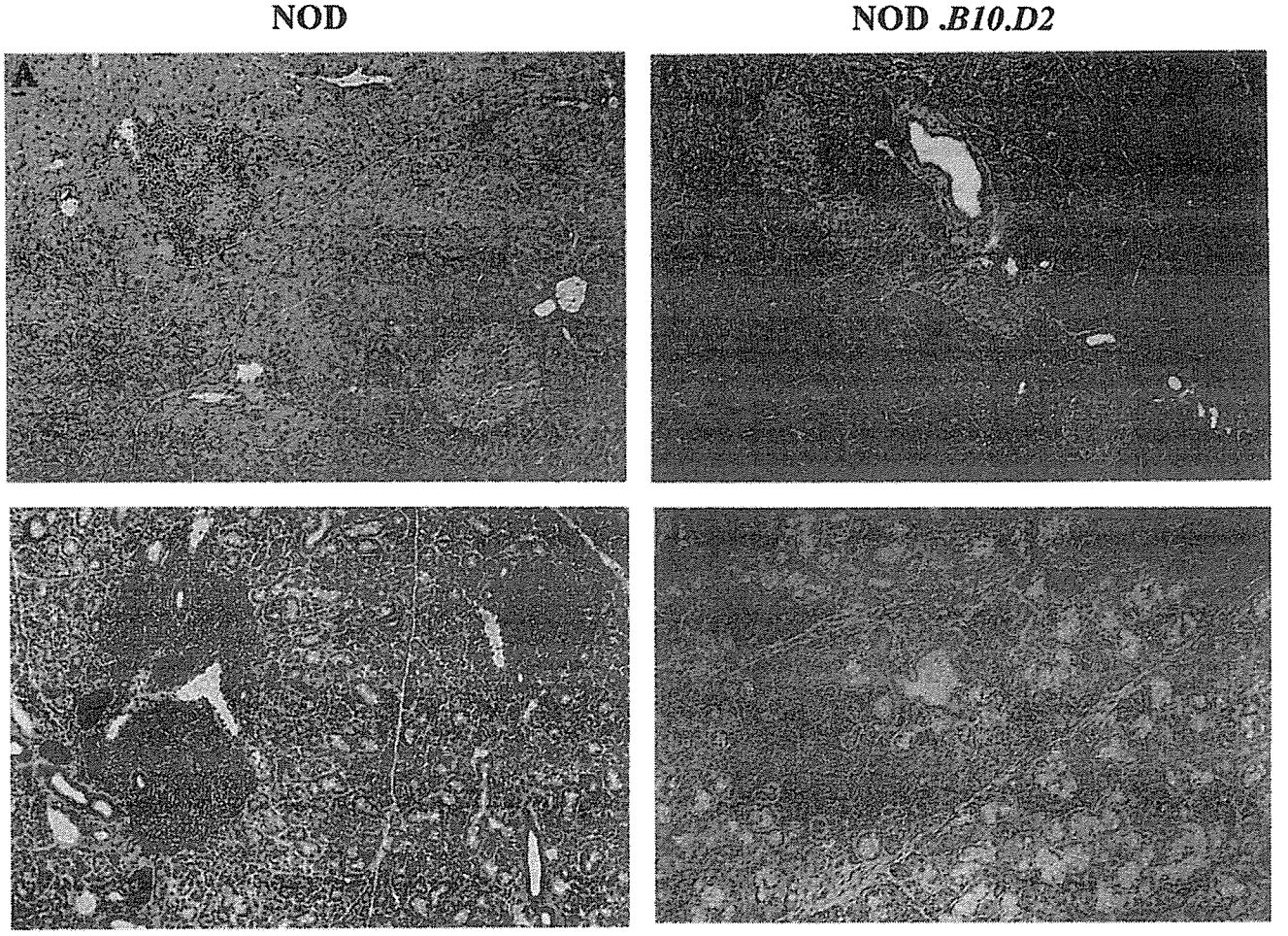


FIG. 3. Histology of exocrine glands from NOD, N5 (NOD.*B10D2*), and N9 (NOD.*B10D2*) female mice at 4 months of age. (A) Massive infiltration by mononuclear cells in the pancreas islets of NOD mice; (B) no infiltrating cells in the pancreas islets of N5 (NOD.*B10D2*) mice; (C) massive infiltration by mononuclear cells in the submandibular glands of NOD mice; and (D) moderate to massive infiltration by mononuclear cells in the submandibular glands of N5 (NOD.*B10D2*) mice; (E) massive infiltration by mononuclear cells in the submandibular glands of N9 (NOD.*B10D2*) female mice. Tissues were stained with hematoxylin and eosin. Magnification, × 100.

MHC serological typing. Spleen cells were dissociated from NOD and MHC-recombinant N5 NOD and N9 NOD mice and incubated with monoclonal antibodies (mAbs) determined to react with MHC class I and class II molecules. Fluorescein isothiocyanate- and phycoerythrin-conjugated goat anti-mouse mAb H-2K^d (SF-1.1.1) reacted in cells from both NOD and B10.D2 mice, and mAb I-E^k (14-4-4S) and mAb H-2D^d (34-5-8S) reacted in cells from B10.D2 mice. mAb I-E^k was used for detection of *I-E^d* expression because it cross-reacted with the I-E^d molecule. After being incubated with these mAbs for 45 min at 4°C, the cells were washed and then analyzed by flow cytometry (fluorescence-activated cell sorting [FACS]) (Becton Dickinson).

Histology. Pancreas specimens and submandibular glands were frozen in OCT compound. Tissue sections (5 μm) were stained with hematoxylin and eosin and were then examined for evidence of mononuclear cell inflammation. Histological observations and photomicrography were performed by using an Olympus BX50WI microscope (Olympus Inc., Tokyo, Japan).

Statistical analysis. A Kaplan-Meier cumulative survival test was used to compare the incidence of diabetes. Comparative analyses were performed by analysis of variance. A P value of < 0.05 was considered statistically significant for two-tailed comparisons. All statistical analyses were performed using StatView software for the Macintosh operating system.

RESULTS

Generation of backcross and intercross of NOD.*B10.D2* strain and assessment of diabetes and salivary flow rate. BC4 and BC8 heterozygous mice (*Mhc*: *d/d* at *K*, *d/g7* at *A* and *E*,

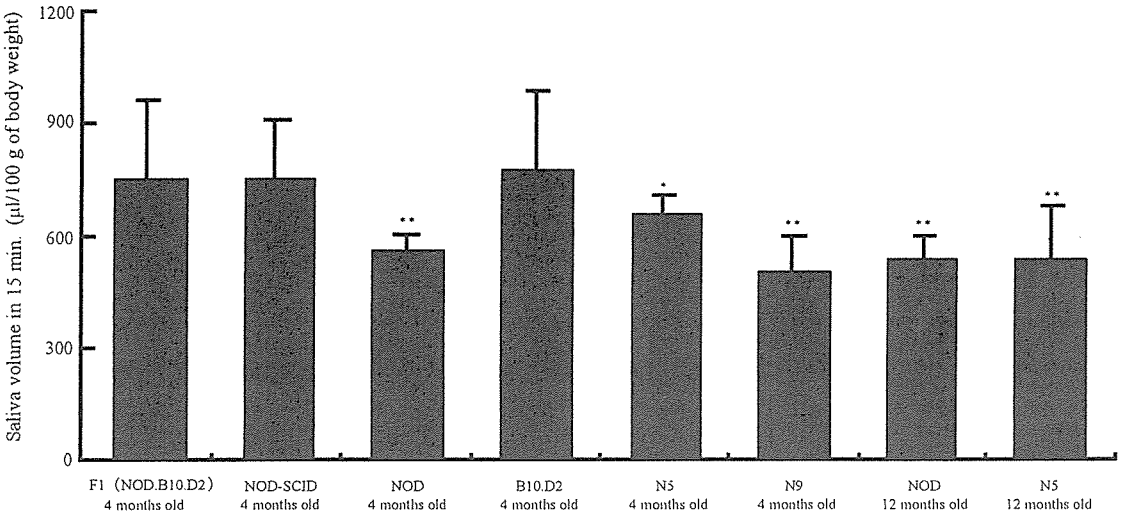


FIG. 4. Salivary flow rates for various mouse strains. The total volume of secreted saliva per 100 g of body weight for NOD, N5 (NOD.*B10.D2*), and N9 (NOD.*B10.D2*) female mice was reduced compared to that for NOD-*scid*, F_1 (NOD.*B10.D2*), and B10D2 female mice at 4 months of age. Data are expressed as the means ± SDs of the results for 12 mice per strain. *, $P < 0.05$; **, $P < 0.001$.

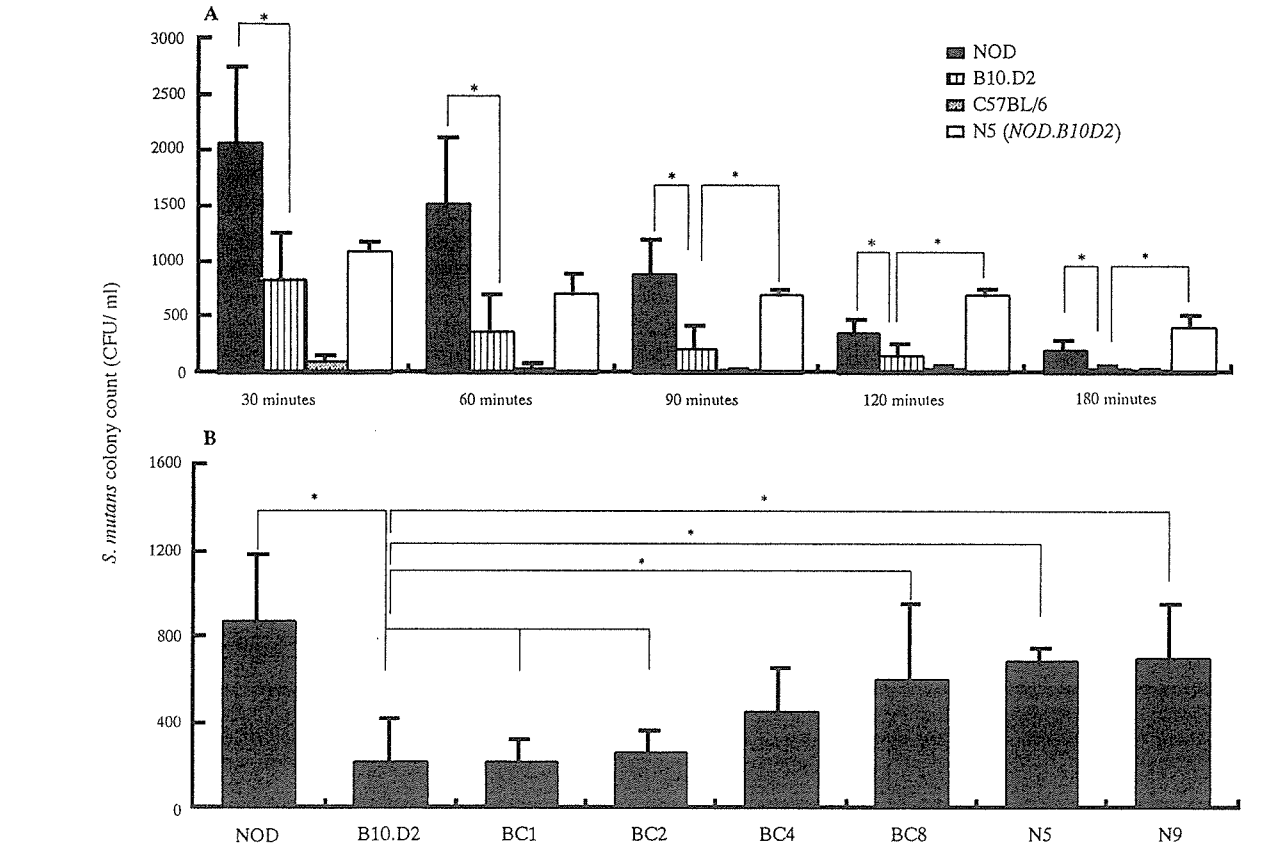
and *d/b* at *D*) were intercrossed to produce N5 (NOD.*B10.D2*) and N9 (NOD.*B10*.D2) generation mice. The N5 (NOD.*B10.D2*) and N9 (NOD.*B10.D2*) intercross animals were typed for the *Mhc* haplotypes and divided into three groups (NOD type, heterozygous, and homozygous). Further, the expression of MHC class I K^d, class II *I-*E^d, and class I D^d in the backcross and intercross N5 (NOD.*B10.D2*) mice was confirmed serologically by FACS analysis (Fig. 1). Mice that were homozygous for B10.D2 *Mhc* in the fourth and eighth backcross generations (BC4 and BC8) were used to establish the N5 (NOD.*B10.D2*) and N9 (NOD.*B10.D2*) strains and were then maintained by brother-sister mating. Replacement (homozygous recombination) of the MHC class II (*A E*) and class I *D* regions prevented the development of diabetes (78% of NOD type and 8% of N5 heterozygous strains developed diabetes). However, the incidence of insulitis in the N5 (NOD.*B10.D2*) strain (grade 0, 92%; grade 1, 8%; grade 2, 0%; grade 3, 0%; and grade 4, 0% at 6 months of age) was significantly different from that in the NOD type mice, and no insulitis was observed in F_1 mice (Fig. 2B). Histological examination of the pancreas specimens showed only slight insulitis in the N5 (NOD.*B10.D2*) mice compared with that in the NOD colony (Fig. 3), while examination of the submandibular glands showed a strong infiltration of lymphocytes in the N5 (NOD.*B10.D2*) and N9 (NOD.*B10.D2*) strains (Fig. 3). Thus, replacement of the NOD MHC class II *I-A* and class I *D* regions with the B10-D2 MHC Class II *I-A* and Class I *D* regions prevented the development of diabetes and insulitis but not SS in N5 (NOD.*B10.D2*) and N9 (NOD.*B10.D2*) generation mice.

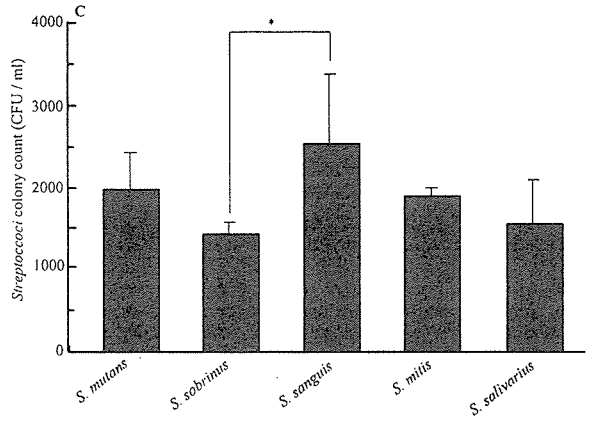
As shown in Fig. 4, the total volume of secreted saliva after stimulation by a secretagogue was significantly lower for NOD mice and N5 and N9 (NOD.*B10.D2*) mice at 4 and 12 months of age than for the F_1 (NOD.*B10.D2*), NOD-*scid*, and B10.D2 mice at 4 months of age. However, there was no significant difference between NOD and N9 (NOD.*B10.D2*) mice at 4 or 12 months of age and only a slight difference between NOD and N5 (NOD.*B10.D2*) mice at 4 months of age.

To assess the incidence of SS, sialadentis was analyzed histologically for those mice that presented a decreasing volume of secreted saliva, as shown in Fig. 4. The incidence of SS with both decreasing saliva and sialadentis was 10 of 12 (83%), 10 of 12 (83%), 9 of 12 (75%), 10 of 12 (83%), and 10 of 12 (83%) in 4-month-old NOD, 12-month-old NOD, 12-month-old N5 (NOD.*B10.D2*), and 12-month-old N9 (NOD.*B10.D2*) mice, respectively. However, sialadentis was not observed in the control 12-month-old B10.D2 and F1 (NOD.*B10.D2*) mice.

Adhesion of oral streptococci to tooth surfaces. As shown in Fig. 5, *S. mutans* adhesion results revealed that bacteria adhered in higher numbers to the tooth surfaces of N5 (NOD.*B10.D2*) and N9 (NOD.*B10.D2*) mice than to those of the control B10.D2, NOD, and C57BL/6 mice at various time points. After the adhesion phase was extended from 90 to 180 min, there was a decrease in biofilm growth (Fig. 5A); however, a large number of bacteria remained on the tooth surfaces of the mice. *S. mutans* adhesion for N5 (NOD.*B10.D2*) mice (407.0 ± 93.0 CFU/ml) and NOD mice (203.0 ± 75.0 CFU/ml) was significantly higher than that for the control B10.D2 and C57BL/6 mice at 180 min after inoculation ($P < 0.05$) (Fig. 5A). Further, distinct differences among N5 (NOD.*B10.D2*), NOD, and B10.D2 mice were observed at 90 min after inoculation. Therefore, bacterial samplings in later experiments with streptococcal inoculation were performed at 90 min. Significantly greater numbers of *S. mutans* bacteria (879.0 ± 302.6, 600.4 ± 351.6, 691 ± 151, and 702.0 ± 205.5 CFU/ml) adhered to the tooth surfaces of NOD, BC8, N5 (NOD.*B10.D2*), and N9 (NOD.*B10.D2*) mice, respectively, than to those of B10.D2 (216.0 ± 203.0 CFU/ml), BC1 (206.0 ± 98.0 CFU/ml), and BC2 (256.0 ± 103.0 CFU/ml) mice (Fig. 5B).

S. mutans, *S. sanguis*, *S. sobrinus*, *S. salivarius*, and *S. mitis* were also inoculated into the oral cavities of N5 (NOD.*B10.D2*) mice (Fig. 5C). The CFU of *S. sanguis* showed that it had the highest level of adhesion among these streptococci. Further, *S. mutans* and *S. mitis* were found in greater numbers than *S. sobrinus* and *S. salivarius*, though the differences





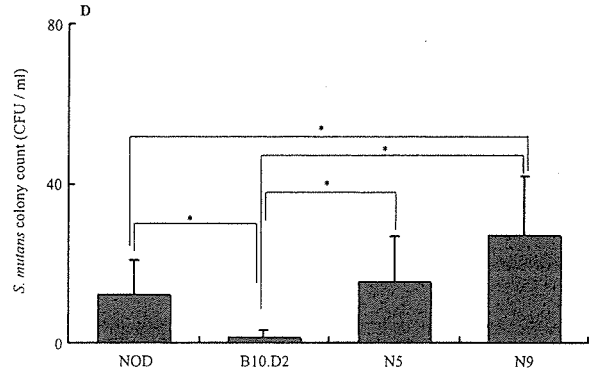


FIG. 5. Adhesion of oral streptococci on tooth surfaces of mice. (A) Adhesion of *S. mutans* to the tooth surfaces of NOD, B10.D2, N5 (NOD.*B10.D2*), and C57BL/6 female mice at 4 months of age at different time points. CFU were determined in Mitis-Salivarius agar plates containing 0.02 M bacitracin after 48 h of anaerobic incubation at 37°C. (B) Adhesion of *S. mutans* for NOD, B10.D2 BC1, BC4, BC2, BC8, N5 (NOD.*B10.D2*), and N9 (NOD.*B10.D2*) female mice at 8 months of age 90 min after inoculation. CFU were determined in Mitis-Salivarius agar plates containing 0.02 M bacitracin after 48 h of anaerobic incubation at 37°C. (C) Adhesion of *S. mutans*, *S. sobrinus*, *S. sanguis*, *S. mitis*, and *S. salivarius* for N5 (NOD.*B10.D2*) female mice at 8 months of age 90 min after inoculation. CFU were determined in Mitis-Salivarius agar plates after 48 h of anaerobic incubation at 37°C. (D) Adhesion of *S. mutans* for NOD, B10.D2, N5 (NOD.*B10.D2*), and N9 (NOD.*B10.D2*) female mice at 8 months of age 24 h after inoculation. Data were obtained from three independent experiments with 15 mice in each strain, and values are expressed as the means ± SDs of the results. *, $P < 0.05$.

were not statistically significant (Fig. 5C). After the adhesion phase was extended from 180 min to 24 h, there was a decrease in *S. mutans* adhesion on the tooth surfaces of the mice (Fig. 5D); however, a significant number of bacteria remained on the tooth surfaces of NOD (17.0 ± 12.0 CFU/ml), N5 (15.0 ± 11.2 CFU/ml), and N9 (31.0 ± 16.0 CFU/ml) mice compared to that for B10.D2 (1.0 ± 2.0 CFU/ml) mice (Fig. 5D).

DISCUSSION

Previous studies of labial salivary glands have suggested that MHC molecules are genetically associated with SS in humans (10, 23, 27). Class II *HLA* antigen is expressed on SS salivary

gland epithelial cells but not in normal salivary gland cells (8, 20). Other results have also demonstrated that there is no single class II allele associated with primary SS among different ethnic groups (14), though there is no direct evidence that the MHC class II gene confers susceptibility to the development of primary SS. Recently, a report by Robinson et al. indicated that the unique NOD MHC class II *I-A*^{g7} is not essential for exocrine tissue autoimmunity in NOD mice (31). In our experiments, we also found that recombination of the region containing MHC class II and class I *D* derived from B10.D2 mice could not prevent development of salivary gland lymphocytic infiltration in NOD mice. However, such a recombination was considered to be essentially responsible for the progression of lymphocytic infiltration in the pancreas. These findings suggest that immunoreactivity to the auto antigen in the N5 (NOD.*B10.D2*) and N9 (NOD.*B10.D2*) mice with replacements of the MHC class II (A^d E^d) and class I D^d regions, respectively, is associated with an immune function that has a role in inflammation of the pancreas islet rather than the development of SS and that separate genes contribute to the development of IDDM and SS in NOD mice.

It has also been reported that salivary components play important roles in controlling microbial colonization in the oral cavities of individuals with SS or dry mouth (37). SS may lead to qualitative and quantitative changes in the protective salivary films or pellicles that coat hard and soft tissues (5, 6), and the loss of enamel or cemental protective pellicles could result in an increase in dental caries and periodontitis (32). Further, an alteration in the mucosal pellicle may make oral soft tissues more susceptible to desiccation and environmental insult, leading to colonization by opportunistic microflora (21, 24). The *S. mutans* adhesion results seen in the present experiments demonstrated that a significant number of bacteria adhered to the tooth surfaces of NOD, N5 (NOD.*B10.D2*), and N9 (NOD.*B10.D2*) mice compared to that for C57BL/6 and B10.D2 mice (Fig. 5A, B and D). Further, the NOD background gene tended to increase the binding of *S. mutans* to tooth surfaces as the number of backcrosses increased (Fig. 5B). However, NOD mice that develop diabetes are not suitable for studies of long-term infection by oral bacteria, as their average life span is short compared with those of N5 (NOD.*B10.D2*) and N9 (NOD.*B10.D2*) mice.

The ability of oral streptococci to bind to salivary pellicle proteins on the tooth surface is of considerable etiological significance (28, 33), and *S. mutans* and *S. sanguis* are known to be primarily involved with the formation of bacterial flora on teeth. *S. sanguis* and *S. mitis* are early colonizers of the salivary pellicle, while *S. mutans* colonizes later; however, the ability of each to bind to salivary proteins and glycoproteins is strong and important in biofilm development (19, 40). The present results showed a tendency for an increase in affinity of *S. sanguis*, *S. mitis*, and *S. mutans* for the mouse tooth surfaces (Fig. 5C). The affinity of streptococci for the tooth surfaces of patients is considered to be closely related to dry mouth and SS (1–3, 4, 34). Therefore, the N5 (NOD.*B10.D2*) and N9 (NOD.*B10.D2*) mouse strains may be useful animal models, as they presented initial adherence activities of oral streptococci on their tooth surfaces similar to those for humans with dry mouths. They may also be suitable for studies of long-term infection by oral bacteria, as their average life span is long compared to that of NOD mice.

A problem facing in vivo oral biofilm research is the lack of a naturalistic, reproducible, longitudinal monitoring system that would permit the assessment of dry mouth and oral bacterial infection in the same animal throughout the duration of the study. Studies of *S. mutans* infections in mouse oral cavities have been performed by feeding the animals with powdered diet 301 and diet 2000, which contain unnatural amounts of sucrose (1 and 56%, respectively) (13, 38, 43). In other infection studies, mice were provided with either a 5% sucrose diet or a sucrose-free diet, in which the 56% sucrose in diet 2000 was replaced with wheat flour (15, 30). When these methods were used, *S. mutans* was found to produce a larger amount of insoluble glucan in the oral cavities of mice than in normal humans. However, continuous ingestion of food containing such excess amounts of sucrose is unusual (35). Therefore, an experimental system using a generated NOD.*B10.D2* strain may be more useful than those previously reported for the initial adherence of streptococci on tooth surfaces without synthesis of insoluble glucan.

In the present study, the importance of salivary flow for controlling the initial adhesion of oral streptococci in the oral cavity was also demonstrated in the mouse models of SS and dry mouth. We believe that N5 (NOD.*B10.D2*) and N9 (NOD.*B10.D2*) mice, which have a high sensitivity for initial adhesion, may be useful for in vivo biological studies of oral biofilm formation on the tooth surfaces of patients with dry mouth or SS.

ACKNOWLEDGMENTS

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原　著

自立生活高齢者と要介護高齢者の口腔微生物叢の比較

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概要：自立生活高齢者（自立高齢者）と要介護高齢者の口腔微生物叢の差異を検討する目的で，調査開始時と1年後の2時点において歯垢中の微生物の検出を試みた．さらに，これらの微生物の検出状況と口腔状態，および口腔衛生習慣との関連性についても検討し，以下の結果を得た．

自立高齢者（41名）における調査開始時の口腔微生物検出種数は，平均3.0±1.3種，1年後で平均4.3±1.7種であった．要介護高齢者（20名）における調査開始時の口腔微生物検出種数は，平均3.3±1.5種，1年後では平均3.5±1.6種であった．自立および要介護高齢者の口腔清掃状況はおおむね良好であったが，自立高齢者，要介護高齢者ともに好気性微生物では *Candida* sp.，嫌気性微生物では *Prevotella melaninogenica* が最も高率に検出された．さらに両群ともに，好気性微生物に比べ嫌気性微生物で検出率が高いものが多く認められた．さらに，義歯を装着している要介護高齢者で *Candida* sp. の検出率が高かった．また，自立高齢者では1日の義歯洗浄回数が少ない者ほど *Candida* sp. と *P. melaninogenica* の検出率が高くなる傾向がみられた．

索引用語：高齢者，要介護高齢者，口腔微生物叢

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緒　言

近年，口腔疾患と全身疾患との関連性が注目されており，口腔状態と消化器系疾患[1]，歯周疾患と循環器系疾患[2]や糖尿病[3]との関連性について調べた報告がみられる．平成8年には厚生省（現・厚生労働省）が健康政策調査研究事業の一環として「口腔保健と全身的な健康状態との関係について」をテーマとする研究プロジェクトをスタートさせ，学際的な面からの検討も進められている．

一方，わが国は急速に高齢社会に突入したことから，高齢者に対する保健・医療対策が急務となっている．厚生労働省の人口動態統計によると，1980年以降65歳以上の高齢者では，肺炎による死亡が全死因の第4位となっているが，直接死因としては肺炎および感染症が半数を占めるとの報告もみられる[4]．さらに，肺炎で死亡する人のおよそ92%が65歳以上の高齢者であることが示されている[5]．Granton ら[6]と Niederman[7]は，60歳以上の者では自立生活者においても，施設入所者においても，肺炎予防が健康維持に重要であることを報告している．さらに高齢者や要介護高齢者にみられる肺炎では，嚥下性肺炎が高い割合を示していることから[7,8]，その対策は高齢社会における重要課題の1つになってきている．肺炎の起炎細菌として *Streptococcus pneumoniae*，*Staphylococcus aureus*，*Haemophilus influenzae*，*Klebsiella pneumoniae* などが知られている[9]．これらの細菌は口腔常在菌としてみられる場合も多いことから[10-12]，嚥下性肺炎の発症と口腔細菌との関連が調べられている[12-14]．

弘田ら[15]は特別養護老人ホーム入所者を対象に，5カ月間にわたり口腔ケアを実施し，咽頭からの総細菌数，レンサ球菌数およびブドウ球菌数の変動を観察した．その結果，歯科医師と歯科衛生士による口腔ケアはいずれの細菌数も減少させたことから，積極的な専門家による口腔ケアは口腔感染症の予防のみならず，呼吸器感染症を含む種々の全身感染症を軽減する疾病予防につながる可能性を示している．米山[16]は，同じく特別養護老人ホームに入所している要介護高齢者に対して2年間の口腔ケアを実施し，その前後における肺炎発症率を比較した．その結

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果，口腔ケア後では発熱および肺炎の発症率が低下し，肺炎予防における口腔ケアの効果を明らかにしている．

Margaret ら[17]は55歳以上の358名について，肺炎発症と医・歯科学的リスクファクターとの関連性を調べた．9年間の追跡調査期間に50名が肺炎を発症しており，種々のリスクファクターに対するロジスティック回帰モデルを用いた分析を行った結果，口腔健康状態の改善が肺炎の発症率低下をもたらすことを示している．さらに泉福ら[18]は要介護高齢者において，寝たきりの程度と口腔起炎菌の検出状況との関連性を明らかにし，*Klebsiella pneumoniae* や *Pseudomonas* sp. を指標とした口腔ケアの重要性を報告している．このような研究結果は，要介護高齢者における口腔起炎菌の適切なコントロールが，肺炎予防をはじめとする健康管理に効果的であることを示している．

一般に高齢者は，老化や種々の全身性疾患を有する場合が多いことから，感染に対する防御機能が低下していると考えられる[19]．すなわち，高齢者における口腔ケアは介護の必要性の有無にかかわらず重要となってくる．従来，要介護高齢者の好気性細菌や真菌を調べた報告はみられるが，介護を必要としない自立生活高齢者（以後自立高齢者とする）を対象とした研究は少ない．特に嫌気性細菌にも着目し，自立高齢者と要介護高齢者における検出状況を同時に比較した報告はみることができない．

本研究では，自立高齢者と要介護高齢者の口腔における好気および嫌気性微生物を調べる目的で，調査開始時と1年後の2時点において，高齢者で口腔気道系感染症の起炎菌となることの多い菌種を中心に検出を試みた．さらに，これら微生物の検出状況と口腔状態，および口腔衛生習慣との関連性についても検討した．

対象者および方法

1. 対象者

自立高齢者は埼玉県某村在住の65歳以上の男女計41名（男22名，女19名）で，同村保健センターにおいて実施した健康調査を受診した者である．調査開始時の年齢は66～86歳（平均年齢73.4±5.6歳）であった．要介護高齢者は，東京都内の老人病院または特別養護老人ホームに入院/入所している男女20名で，調査開始時の年齢は64～96歳（平均年齢77.1±9.4歳）であった．要介護の程度は対象者全員が「準寝たきり状態[19]」であった．本研究を実施するにあたって，対象者や介護者に対して研究内容の十分な説明を行い，同意を得た．

2. 方法

1）口腔診査

対象者の口腔状況を把握する目的で，現在歯数と歯垢の付着程度（PlI：Plaque index）を調べた．歯垢の付着判定はSilness と Löe の判定基準[20]に準じたが，測定部位は現在歯すべてとして，歯数あたりの平均値を算出した．なお，無歯顎者は除外した．

2）口腔微生物の検出

口腔微生物検出のための試料（歯垢）採取は，滅菌綿棒（シードスワブ1号，栄研）を用いて対象者の左側上顎第二小臼歯，第一大臼歯および第二大臼歯相当部位の頬側歯頸部を5回拭った後に，綿棒を180度回転させてさらに同部位を5回拭うことにより行った[18]．対象部位に歯がない者については，相当部位付近を前述の方法にて拭った．その後ただちに，綿棒に付属している検体保存輸送用培地に移した．検体採取後6時間以内に以下に示す各種培地を用いて，5% CO_2 培養および嫌気培養（AnaeroPack・ケンキ，三菱ガス化学）を開始した．①コロンビア5%ヒツジ血液寒天培地〔Nippon Becton Dickinson Company（BD）〕，②BTB培地（BD），③チョコレートⅡ寒天培地（BD），④OPA ブドウ球菌寒天培地（BD），⑤PASA 培地（BD），⑥ブルセラ血液寒天培地（栄研），⑦サブロー培地（BD）．

24～48時間の初代分離培養を行った後，コロニーを釣菌し，以下に示す確認培地および同定キットを用いて，起炎菌を中心に目的菌の同定を行った．①MRSA（methicillin-resistant *Staphylococcus aureus*）およびMSSA（methicillin-susceptive *Staphylococcus aureus*）：PS ラテックス（栄研）・ウサギプラズマ（栄研）・MRSA スクリーニング培地（BD），②*Pseudomonas aeruginosa*，*Klebsiella pneumoniae*，*Serratia marcescens*，*Moraxella*（*Branhamella*）*catarrhalis* およびほかのグラム陰性桿菌：VITEK〔bioMerieux VITEK Japan（BVJ）〕，③β溶連菌：セロアイデンストレプトキット（栄研）・rapid ID 32 strep API（BVJ），④*Streptococcus pneumoniae*：肺炎球菌鑑別用ディスク/タキソPディスク（BD）・ストレプト（BVJ），⑤*Haemophilus infuluenzae*：ヘモフィルスID4分画（BD），⑥*Candida* sp.：カンジダチェック（ヤトロン），なお，嫌気性菌の同定にはRap ID ANA Ⅱ（アムコ，東京）を用いて行った．

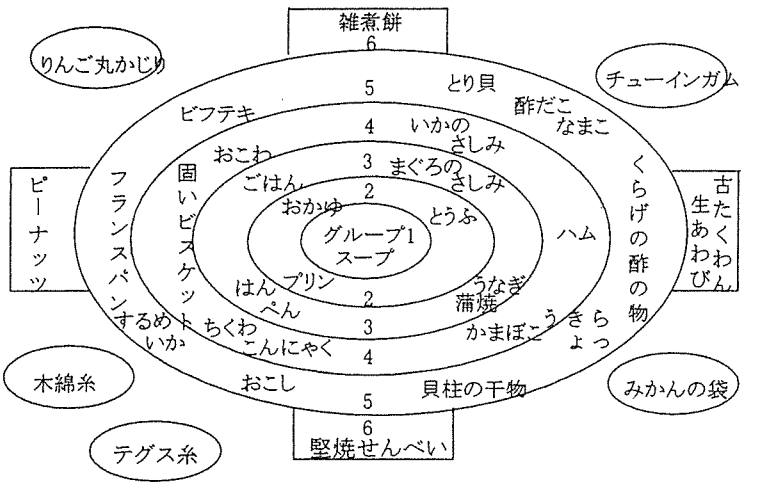
3）口腔衛生習慣に関するアンケート調査

対象者の口腔衛生習慣を調べる目的で，調査開始から1年間の状況を中心に，質問票（図1）によるアンケート調査を行った．調査は聞き取り方式で，対象者に対して実施したが，要介護高齢者は必要に応じて介護者の協力

[B]<5>　間食・夜食は控えていますか。
1. 控えている　2. あまり控えていない　3. 控えていない

[B]<9>　甘い物は好きですか。
1. 好き　2. 普通　3. 嫌い

[B]<10>　どのくらいの硬さの食材を食べられますか。



[C]<1>　歯を磨くのは誰ですか。(あてはまるもの全てお答え下さい。)
1. 自分　2. 介護者(家族をのぞく)　3. 家族　4. その他(　　)

[C]<2>　歯をいつ磨きますか。(あてはまるもの全てお答え下さい。)
1. 朝起きてすぐ　2. 朝食後　3. 昼食後　4. 夕食後　5. 夜寝る前
6. その他(　　)

[C]<3>　義歯を使用してますか。
1. 持っていない　2. 食事中はずす　3. 常時装着　4. 食事中のみ装着
5. 必要なし(現在歯数20本以上ある)

2. 3. 4. の方のみお答えください。
[C]<4>　義歯をどのくらいの間隔で洗いますか。
1. 1回／1日　2. 2〜3回／1週間　3. 1回／1週間　4. 洗わない
5. その他(　　)

図1　口腔衛生習慣に関するアンケート用紙（抜粋）

を得た．なお，咀嚼能率は山本式咀嚼能率指数判定表[21]を用いて求めた．

3. 統計学的解析

平均値の差の検定にはt検定，微生物の検出率の比較には割合の差の検定，検出状況と口腔衛生習慣との関連についてはχ^2検定を行い，それぞれ危険率5%を基準に有意差または関連性ありと判定した．

結　果

1. 対象者の年齢分布

図2に対象者の年齢分布を示した．自立高齢者，要介護高齢者ともに70〜74歳が最も多かった．

2. 口腔診査結果

表1に対象者の現在歯数およびPIIを示した．調査開始時の自立高齢者における現在歯数は，5本以下が全体の51.2%を占めていた．さらに1年後の現在歯数は対象

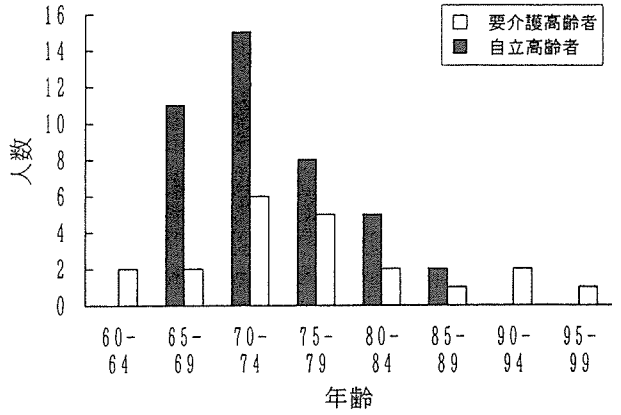


図2　対象者の年齢階級別分布

表1　対象者の現在歯数とPII

		現在歯数	PII
自立高齢者	調査開始時	9.9 ± 10.5	1.1 ± 0.9
(n=41)	1年後	9.6 ± 10.7	1.1 ± 0.6
要介護高齢者	調査開始時	10.1 ± 7.9	1.1 ± 1.1
(n=20)	1年後	9.9 ± 7.8	0.9 ± 0.8

平均値±標準偏差

者個人においても明らかな変化は認められなかった．これに対して，調査開始時の要介護高齢者における現在歯数は，5本以下が全体の40.0%を占めていた．1年後でも自立高齢者と同様に，対象者個人においても明らかな変化は認められなかった．歯垢付着状態を示すPIIは，両群ともに調査開始時と1年後のPIIに有意差はみられなかった．さらに各時点におけるPIIについても，自立高齢者と要介護高齢者間で有意な差は認められなかった．

3．口腔微生物の検出結果

1）自立高齢者

図3aとbに，自立高齢者における口腔微生物の検出結果を示した．1人あたりの検出菌種数は調査開始時が1〜7種で平均3.0±1.3種であったのに対し，1年後では1〜8種で平均4.3±1.7種と有意に増加していた（$p<0.01$）．

好気性微生物については図3aに示したように，調査開始時では10菌種が確認された．最も多くの対象者から検出されたのは*Candida* sp.で，検出率は26.8%であった．次いで*Haemophilus parainfluenzae*（23.9%），*Bacillus* sp.（12.0%），*Enterobacter cloacae*（12.0%）の順であった．これらの微生物の検出率は1年後においてもほぼ同様で

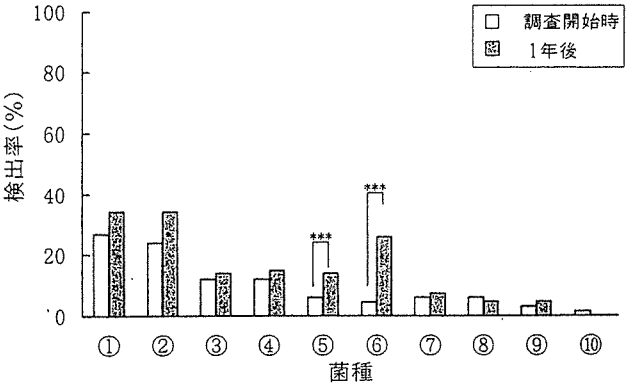


図3a　自立高齢者における好気性微生物の検出率
***：$p<0.001$（調査開始時と1年後との比較）
① *Candida* sp.
② *Haemophilus parainfluenzae*
③ *Bacillus* sp.
④ *Enterobacter cloacae*
⑤ *Acinetobacter calcoaceticus*
⑥ *Corynebacterium* sp.
⑦ *Klebsiella pneumoniae*
⑧ *Klebsiella oxytoca*
⑨ MSSA (methicillin-sensitive *Staphylococcus aureus*)
⑩ *Enterobacter aerogenes*

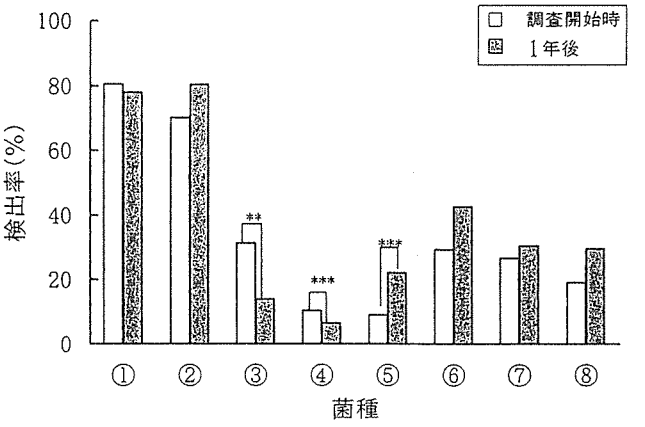


図3b　自立高齢者における嫌気性微生物の検出率
：$p<0.01$，*：$p<0.001$（調査開始時と1年後との比較）
① *Prevotella melaninogenica*
② *Capnocytophaga* sp.
③ *Prevotella denticola*
④ *Clostridium* sp.
⑤ *Prevotella intermedia*
⑥ *Precotella oris*
⑦ *Fusobacterium nucleatum*
⑧ *Fusobacterium necrophorum*

あったが，*Acinetobacter calcoaceticus* と *Corynebacterium* sp. の検出率は，それぞれ調査開始時に比べ有意に高値であった（$p<0.001$）．また，1年後では1名（2.4%）から *Pseudomonas* sp. が検出された．

嫌気性微生物の検出結果を図3bに示した．調査開始時には8菌種が確認されたが，好気性微生物に比べて検出率が高いものが多かった．検出率が30%を超えたものは，*Prevotella melaninogenica*（80.6%），*Capnocytophaga* sp.（70.2%），*Prevotella denticola*（31.3%）であった．1年後の検出率は，*Prevotella denticola* と *Clostoridium* sp. が調査開始時に比べ有意に低値を示し（前者：$p<0.01$，後者：$p<0.001$），*Prevotella intermedia* は有意に高値であった（$p<0.001$）．

2）要介護高齢者

図4aとbに要介護高齢者における口腔微生物の検出結果を示した．1人あたりの検出菌種数は調査開始時，1年後ともに1～8種であり，前者が平均3.3±1.5種，後者が平均3.5±1.6種であった．

好気性微生物については図4aに示したように，調査開始時では自立高齢者で確認された10菌種に加え，*Stenotrophomonas maltophilia* と MRSA が検出された．最も多くの対象者から検出されたのは自立高齢者と同じく *Candida* sp. で，検出率は80.0%であった．次いで *Corynebacterium* sp.（25.0%），*Enterobacter cloacae*（20.0%），*Klebsiella pneumoniae*（20.0%）の順であった．また，1名（5.0%）から MRSA が検出された．1年後については MRSA は検出されず，これを除くと調査開始時と同じ菌種が同様の検出率で確認された．

嫌気性微生物の検出結果を図4bに示した．調査開始時では自立高齢者と同種の8菌種が確認された．要介護高齢者においても，好気性微生物に比べて嫌気性微生物で検出率が高いものが多く認められた．検出率が30%を超えたものは，*Prevotella melaninogenica*（90.0%），*Capnocytophaga* sp.（85.0%），*Prevotella oris*（35.0%），*Fusobacterium nucleatum*（30.0%）であった．1年後においても調査開始時と同じ菌種が同様の検出率で確認された．

3）自立高齢者と要介護高齢者における検出率の比較

自立高齢者と要介護高齢者の両群において共通に検出された菌種について，調査開始時と1年後の各時点で検出率の比較を行った．

嫌気性細菌の検出率はいずれの菌種も両群間で有意な差が認められなかった．好気性細菌および真菌では，*Candida* sp., *Corynebacterium* sp., *Klebsiella pneumoniae* および MSSA の検出率が調査開始時あるいは1年後，もしくは両時点において，自立高齢者に比べ要介護高齢者で有意に高かった（表2）．

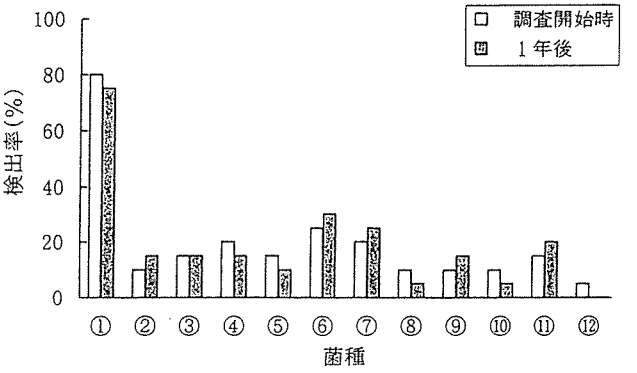


図4a　要介護高齢者好気性微生物の検出率
① *Candida* sp.
② *Haemophilus parainfluenzae*
③ *Bacillus* sp.
④ *Enterbacter cloacae*
⑤ *Acinetobacter calcoaceticus*
⑥ *Corynebacterium* sp.
⑦ *Klebsiella pneumoniae*
⑧ *Klebsiella oxytoca*
⑨ MSSA（methicillin-sensitive *Staphyloccus aureus*）
⑩ *Enterobacter aerogenes*
⑪ *Stenotrophomonas maltophilia*
⑫ MRSA（methicillin-resistant *Staphylococcus aureus*）

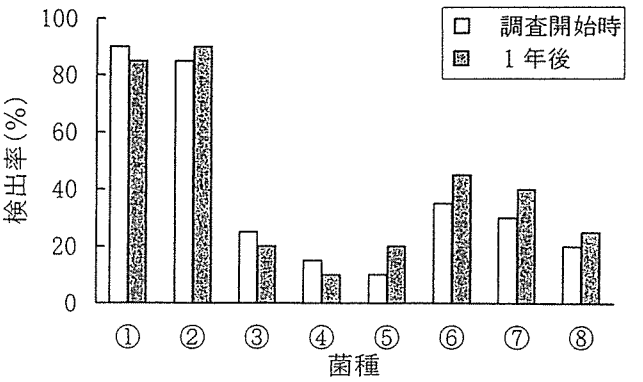


図4b　要介護高齢者の嫌気性微生物の検出率
① *Prevotella melaninogenica*
② *Capnocytophaga* sp.
③ *Prevotella denticola*
④ *Clostridium* sp.
⑤ *Prevotella intermedia*
⑥ *Prevotella oris*
⑦ *Fusobacterium nucleatum*
⑧ *Fusobacterium necrophorum*

4．口腔衛生習慣に関連したアンケート調査の結果

自立高齢者と要介護高齢者の口腔衛生習慣に関連した

アンケート調査の結果を表3と4に示した．自立高齢者では間食・夜食を控えている者が68.3%と多く，歯磨き習慣は全員が1日1回以上行っていた．義歯を常時装着している者の割合は73.2%であったが，比較的硬いものを噛めるが多数を占めていた．

一方，要介護高齢者は施設に入院/入所していることから，種々の生活習慣は自立高齢者と異なるものがみられた．間食・夜食習慣は原則として摂る時間が決められていることから，全員が「控えている」という回答であった．歯磨きは，夜食後の介護者もしくは自身によるブラッシング，および朝昼食後の介護者によるガーゼを用いた口腔内清拭により行われていた．義歯を常時装着している者の割合は50.0%，もっていない者の割合は30.0%であったが，比較的硬いものを噛める者が多かった．また，表中に示していないが，義歯装着者における義歯の洗浄回数については，自立生活者では「毎日洗浄する」が30名中7名（23.3%），「週に2〜3回」が13名（43.3%），「週に1回」が10名（33.3%）であった．一方，要介護者の義歯洗浄は介護者により毎日行われていた．

5．口腔微生物の検出結果と口腔衛生習慣との関連性

自立高齢者における歯磨き回数と検出菌種数との関連について表5に示したが，両者間に有意な関連は認められなかった．なお，前項で示した通り，要介護高齢者では全員が同一の口腔清掃方法を実施していたので，検討を行わなかった．さらに，自立高齢者と要介護高齢者について義歯装着の有無と検出菌種（1年後）との関連，および自立高齢者について義歯の洗浄回数と検出菌種（1年後）との関連をそれぞれ検討した．検討菌種は調査開始時および1年後ともに検出率が高かった好気性および嫌気性微生物各2種と，調査開始時と1年後の検出率に有意差が認められたものとした．

義歯装着の有無と微生物の検出結果との関連を，表6aとb（自立高齢者）および表7aとb（要介護高齢者）にそれぞれ示した．自立高齢者では，いずれの菌種についても義歯装着の有無と間に有意な関連性は認められなかった．

要介護高齢者については，義歯装着の有無と*Candida* sp.の検出率との間に有意な関連性が認められ（$p<0.05$），義歯非装着者に比べ装着者で検出率が高かった．ほかの菌種では関連がみられなかった．

自立高齢者における義歯の洗浄回数と検出菌種との関連について，表8a（好気性微生物）とb（嫌気性微生物）に示した．*Candida* sp.および*Prevotella melaninogenica*の2菌種について，それぞれの検出率と義歯洗浄回数との間に有意な関連が認められ（前者 $p<0.05$，後者 $p<0.01$），

表2　口腔微生物の検出率の比較

		調査開始時 (%)		1年後 (%)	
Candida sp.	自	26.8	***	34.3	**
	要	80.0		75.0	
Corynebacterium sp.	自	4.5	**	25.9	
	要	25.0		30.0	
K. pneumoniae	自	6.0	*	7.4	*
	要	20.0		25.0	
MSSA	自	3.0		4.6	**
	要	10.0		15.0	

自：自立高齢者　要：要介護高齢者

*：$p<0.05$，**：$p<0.01$，***：$p<0.001$

表3　自立高齢者の口腔衛生習慣

間食・夜食習慣	控えている	68.3(%)
	あまり控えていない	26.8
	控えていない	4.9
甘味嗜好	好き	53.7
	普通	34.1
	嫌い	12.2
山本式咀嚼能率指数	1点　柔らかい	0.0
	2	0.0
	3	0.0
	4	34.1
	5	12.2
	6点　堅い	53.7
歯磨き習慣	1日1回	41.5
	1日2回	36.6
	1日3回	14.6
	1日4回以上	7.3
義歯の使用状況	もっていない	4.9
	食事中はずす	0.0
	常時装着	73.2
	食事中のみ装着	0.0
	必要なし（現在歯数20本以上）	22.0

数値は小数点第2位を四捨五入したもの

表 4　要介護高齢者の口腔衛生習慣

間食・夜食習慣	控えている	100 (%)
	あまり控えていない	0
	控えていない	0
甘味嗜好	好き	60.0
	普通	30.0
	嫌い	10.0
山本式咀嚼能率指数	1点　柔らかい	5.0
	2	0
	3	5.0
	4	15.0
	5	15.0
	6点　堅い	60.0
歯磨き習慣	介護者による朝昼食後のガーゼによる清拭と夜間の歯磨き	100
義歯の使用状況	もっていない	30.0
	食事中はずす	5.0
	常時装着	50.0
	食事中のみ装着	0
	必要なし（現在歯数20本以上）	15.0

いずれも洗浄回数の少ない者で検出率が高かった．

考　察

1. 口腔状況について

自立高齢者と要介護高齢者の口腔状況を現在歯数とPIIから評価した．平均現在歯数は両群ともに10本程度であったが，5本以下の者が自立高齢者で約半数，要介護高齢者で4割を占めていた．

平成11年の歯科疾患実態調査報告[22]によると，1人平均現在歯数は20～24歳が最も多く28.6本であった．以降，年齢が上がるとともに現在歯数は漸次減少していくが，60から70歳代にかけて急激に減少し，80～84歳で7.4本，85歳以上では4.0本であった．

本研究においては，自立高齢者および要介護高齢者ともに70～74歳が最も多いことから，この年齢階級におけ

表5　自立高齢者における1日の歯磨き回数と検出菌種数との関連

		検出菌種数	
		1～4	5以上
1日の歯磨き回数	1回	7	10
	2回	7	8
	3回以上	6	3

表 6a　自立高齢者における義歯装着の有無と微生物検出結果との関連（好気性微生物）

		Candida sp.		*Haemophilus parainfluenzae*		*Acinetobacter calcoaceticus*		*Corynebacterium* sp.	
		(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)
義歯装着	有	10	20	8	22	3	27	6	24
	無	4	7	6	5	3	8	5	6

(+)：検出，(−)：非検出

表 6b　自立高齢者における義歯装着の有無と微生物検出結果との関連（嫌気性微生物）

		Prevotella melaninogenica		*Capnocytophaga* sp.		*Prevotella denticola*		*Clostridium* sp.		*Prevotella intermedia*	
		(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)
義歯装着	有	23	7	21	9	3	27	2	28	6	24
	無	9	2	11	0	3	8	1	10	3	8

(+)：検出，(−)：非検出

表 7a　要介護高齢者における義歯装着の有無と微生物検出結果との関連（好気性微生物）

		Candida sp.		*Corynebacterium* sp.	
		(+)	(−)	(+)	(−)
義歯装着	有	11	0	4	7
	無	4	5	2	7

(+)：検出，(−)：非検出，*Candida* sp. と義歯装着の有無とに関連性あり（$p < 0.05$）

表 7b　要介護高齢者における義歯装着の有無と微生物検出結果との関連（嫌気性微生物）

		Prevotella melaninogenica		*Capnocytophaga* sp.	
		(+)	(−)	(+)	(−)
義歯装着	有	10	1	8	3
	無	7	2	6	3

(+)：検出，(−)：非検出

表 8a　自立高齢者における義歯洗浄回数と微生物検出結果との関連（好気性微生物）

		Candida sp.		*Haemophilus parainfluenzae*		*Acinetobacter calcoaceticus*		*Corynebacterium* sp.	
		(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)
義歯洗浄回数	毎日	1	5	2	5	0	7	2	5
	2〜3回／週	2	11	2	11	2	11	1	12
	1回／週	7	3	4	6	1	9	3	7

(+)：検出，(−)：非検出，義歯洗浄回数と *Candida* sp. との間に有意な関連性あり（$p < 0.05$）

表 8b　自立高齢者における義歯洗浄回数と微生物検出結果との関連（嫌気性微生物）

		Prevotella melaninogenica		*Capnocytophaga* sp.		*Prevotella denticola*		*Clostridium* sp.		*Prevotella intermedia*	
		(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)
義歯洗浄回数	毎日	2	5	6	1	1	6	0	7	2	5
	2〜3回／週	11	2	7	6	1	12	2	11	1	12
	1回／週	9	1	8	2	2	8	0	10	3	7

(+)：検出，(−)：非検出，義歯洗浄回数と *Prevotella melaninogenica* との間に有意な関連性あり（$p < 0.01$）

る前述の歯科疾患実態調査結果をみると12.7本であった．自立高齢者および要介護高齢者ともに現在歯数が5本以下の者が多いことからも，本研究における自立高齢者および要介護高齢者の現在歯数は，全国平均よりも低値であることが推測された．

PIIについては全国規模の調査結果がないことから，数値に対する相対的な評価はしにくい．しかし，自立高齢者および要介護高齢者のPIIは，調査開始時および1年後ともに「よい」または「普通」に分類されたことから[23]，両群ともに現在歯数は少ないものの，歯垢の付着状況からみた口腔清掃状況はおおむね良好と考えられた．

2．口腔微生物の検出結果および口腔状況について

ヒト口腔内には350種にも及ぶ細菌をはじめとする微生物が生息していると考えられている[24]．これら口腔微生物叢のうち，う蝕や歯周疾患の発症に関連するものについては，膨大な数の研究が行われてきている．

近年，口腔の状態と全身の健康との関連が注目されており，口腔細菌と全身性疾患に関する研究が行われるようになった[1-3]．なかでも，高齢者や要介護高齢者において高い死亡率を示している嚥下性肺炎の発症に口腔細菌が関与しているとの報告は多い[5-10]．こうした研究成果をふまえ，高齢者や要介護高齢者に対する口腔ケアは，単に嚥下性肺炎予防のためだけでなく，全身の健康面からも重要であり，さらにはQOLの向上につながっていくものと認識されるようになってきた[15,16]．

従来，要介護高齢者における嚥下性肺炎について，口腔の好気性細菌や真菌に関する疫学調査が行われているが，嫌気性微生物や自立高齢者について同時に調べた研究はみることができない．

本研究結果から，自立高齢者および要介護高齢者の口腔から，複数種の好気および嫌気性の起炎性微生物が検出された．検出時期は調査開始時と1年後の2時点であったが，両時期において同一の起炎性微生物が検出された．しかし，これらの微生物が持続的に感染しているのか否かについては，さらに検討する必要がある．また，自立高齢者と要介護高齢者における1年後の微生物検出状況の違いについても，本研究では明らかにすることができなかった．現在，本研究の対象者に対して専門的な口腔ケアなどを実施しており，口腔微生物叢の変化について縦断的に検討していく予定である．

また，自立高齢者および要介護高齢者では，好気性微生物に比べ*Prevotella melaninogenica*や*Capnocytophaga* sp.のような嫌気性微生物の検出率が高かったことから，嫌気性微生物による口腔感染のリスクが高まっていることも推測された．嫌気性微生物は，肺炎や心臓疾患などの全身性疾患の発症に関与していることから[25-27]，高齢者においては，好気性微生物とともにこれらの嫌気性微生物の検出状況を指標とした口腔ケアの実施が重要となることが示された．

さらに，要介護高齢者では自立高齢者に比べ，*Candida* sp. *Corynebacterium* sp., *Klebsiella pneumoniae*やMSSAの検出率が高かったことから，肺炎の発症リスクが高い状況にあると考えられる．一方で，歯垢の付着量の指標であるPIIは，自立高齢者と要介護高齢者とで差が認められなかった．したがって，肺炎のリスク判定ならびに口腔ケア効果を評価する際には，歯垢の付着程度よりも歯垢を含む口腔微生物叢について検討することが重要であると考えられた．

3．口腔微生物の検出結果と口腔衛生習慣

口腔微生物と義歯装着との関連については，装着者で*Candida* sp.の感染率が高いとの報告がみられる[28-30]．本研究の自立高齢者における微生物の検出状況は，義歯装着の有無で違いがみられなかった．一方，要介護高齢者における*Candida* sp.の検出率は，義歯装着者が非装着者に比べ高く，従来の報告と一致していた．自立高齢者の1日の歯磨き回数は，1回の者が40%以上を占めており，このことが義歯の装着の有無にかかわらず，微生物の感染を引き起こしているものと推察された．

自立高齢者における義歯の洗浄回数と微生物の検出率との関連は，*Candida* sp.と*Prevotella melaninogenica*において負の相関が認められた．したがって，口腔ケアが十分に行き届かない高齢者では，義歯洗浄を含めた口腔清掃が起炎菌減少に効果的であることが考えられた．

本研究結果から，高齢者の健康管理には口腔からの起炎性微生物の除菌またはコントロールが重要であることが明らかにされた．さらに，高齢者自身によるケアや介護者によるケアだけでは必ずしも十分な効果があがっていないことも推察された．したがって，今後高齢者やその介護者に対して，口腔ケア法を指導・教育して必要性が示された．

今回調べた要介護者については，軽度の痴呆を有する者が2名で，ほかの者は日常生活に支障をきたすような症状などはなかった．痴呆度により口腔衛生行動に違いが生ずることも考えられることから，両者の関連や口腔微生物叢について検討する必要があると考えられた．

結　論

1．調査開始時の現在歯数は5本以下の者が自立高齢者で51.2%，要介護高齢者で40.0%を占めていた．その割合は両群ともに1年後でもほぼ同様であった．