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# Role of gene *E2f1* in susceptibility to bacterial adherence of oral streptococci to tooth surfaces in mice

**N. Matsumoto[1,2], M. A. Salam[2], H. Watanabe[2], T. Amagasa[1], H. Senpuku[2]**

[1]Maxillofacial Surgery, Department of Maxillofacial Reconstruction and Function, Division of Maxillofacial and Neck Reconstruction, Graduate School, Tokyo Medical and Dental University, [2]Department of Bacteriology, National Institute of Infectious Diseases, Shinjuku-ku, Tokyo, Japan

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Dental plaque is composed of a biofilm community of microorganisms on teeth that coats the oral cavity, including attaching to the teeth, and provides a protective reservoir for oral microbial pathogens, which are the primary cause of persistent and chronic infectious diseases. Oral streptococci are pioneering organisms that play an important role in biofilm formation on tooth surfaces as well as being primary causative agents of dental caries. The purpose of this study was to clarify the role of the *E2f1* gene in susceptibility to dry mouth and bacterial adherence of oral streptococci to tooth surfaces in animal model experiments. A mutation of the *E2f1* gene in mice is known to cause enhanced T-lymphocyte proliferation, leading to testicular atrophy, splenomegaly, salivary gland dysplasia, and other systemic and organ-specific autoimmunity. We found a decreased volume of saliva production and protein production rate, along with increased amylase activity, IgA concentration, and mucin 1 concentration in E2F-$1^{-/-}$ mice as compared with the control C57BL/6 mice. Further, we quantified the recolonization of oral streptococci in E2F-$1^{-/-}$ mice and found that a higher number of some oral streptococci were colonized on the teeth of these mice. In particular, following oral ingestion of 1% sucrose in water, the colonization of *Streptococcus mutans* increased in comparison with other streptococci. Our results suggest that the *E2f1* gene may affect susceptibility for oral biofilm formation by streptococci in humans with dry mouth.

Key words: biofilm; dry mouth; E2F-1 knockout mouse; saliva; *Streptococcus mutans*

Hidenobu Senpuku, Department of Bacteriology, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo 162–8640, Japan
Tel.: +81 3 5285 1111, Ext. 2222;
fax: +81 3 5285 1172;
e-mail: hsenpuku@nih.go.jp
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Dental plaque is a complex biofilm community of microorganisms that attaches to the surfaces of teeth, consisting of more than 500 bacterial species in a mature state and providing a reservoir for oral microbial pathogens (8, 19, 26). The initial colonizers of freshly cleaned enamel surfaces are predominantly streptococci (24), which account for approximately 20% of the total number of salivary bacteria (18). Oral streptococci are pioneering organisms that play an important role in biofilm formation on tooth surfaces as well as being primary causative agents of dental caries (8, 13, 17). The ability of oral streptococci to bind to the salivary pellicle on the tooth surface protein is of considerable etiologic significance (6, 23, 30), and *Streptococcus mutans* and *Streptococcus sanguis* are known to be primarily involved when bacterial flora forms on tooth surfaces. In addition, *S. sanguis* and *Streptococcus mitis* are early colonizers of the salivary pellicle, while *S. mutans* colonizes later on. The ability of each to bind to salivary proteins and glycoproteins is strong and important in biofilm development (15, 39).

The mechanical forces of salivary flow and tongue movement tend to dislodge and expel bacteria from tooth surfaces and the oral cavity (3, 4). It has been proposed that streptococci bind the salivary component receptors via adhesins present on the cell wall. In addition, there is direct evidence that streptococcal cell wall components mediate adherence to various salivary receptors (5, 23, 29, 31, 33). Their importance in controlling microbial colonization in the oral cavity has also been well demonstrated in individuals with diabetes mellitus, Sjögren's syndrome, and dry

mouth, who suffer from a rapid overgrowth of biofilm and rampant caries, making them highly susceptible to oral infections (1, 2).

Members of the E2F transcription factor family (*E2f1–E2f5*) are important regulators of cell proliferation, differentiation, and apoptosis. The best characterized member of the E2F family is *E2f1* (9, 11, 36), which controls the initiation of DNA synthesis and subsequent transition of cells from the G0/G1 to S phase of the cell cycle (14, 22). Several recent studies have demonstrated that a mutation of the *E2f1* gene in mice causes enhanced T-lymphocyte proliferation, leading to testicular atrophy, splenomegaly, salivary gland dysplasia, and other types of systemic and organ-specific autoimmunity (7, 16, 20, 28, 42, 44). We propose E2F-1 to be a host defense regulator acting against oral pathogens, and attempted to clarify the role of the *E2f1* gene in susceptibility to dry mouth and the initial adhesion of oral streptococci on tooth surfaces in animal model experiments with E2F-1$^{-/-}$ and wild type mice.

## Material and methods

### Bacterial strains and culture conditions

The streptococci strains used in this study were *S. mutans* MT8148*, S. sanguis* ATCC 10556, *Streptococcus salivarius* ATCC 9759*, S. mitis* ATCC 6249, and *Streptococcus sobrinus* 6715. All bacteria were grown in an atmosphere of $H_2$ and $CO_2$ (GasPack, Becton/Dickinson, Sparks, MD) in Brain Heart Infusion broth (BHI, Difco Laboratory, Detroit, MI) at 37°C.

### Animals

E2F-1$^{-/-}$ and NOD/LtJ mice were purchased from The Jackson Laboratory (Bar Harbor, ME), B10.D2 and C57BL/6 mice came from Japan SLC (Shizuoka, Japan), and 129SV mice were provided by Atsuo Ogura, Department of Veterinary Science, National Institute of Infectious Diseases. NOD mice, which naturally develop Sjögren's syndrome and dry mouth, were used to compare the susceptibility for adherence of oral streptococci with E2F-1$^{-/-}$ mice. C57BL/6 and 129SV mice were parental lines to produce E2F-1$^{-/-}$ mice (7) and were used as control mice in saliva and bacterial inoculation experiments. B10.D2 mice having different background genes from E2F-1$^{-/-}$ mice and which did not present salivary gland abnormalities were also used as control mice. All were 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, UK) and diabetes was confirmed to be positive by blood glucose measurements. All mice were provided with sterile food and water, and were used in the experiments at 16–24 weeks of age.

### Bacterial sampling and colony-forming unit (CFU) counting

Bacterial inoculation, sampling and CFU counting were performed using procedures and conditions described previously (32). All oral streptococci were cultured in BHI broth overnight and then washed twice with sterile phosphate-buffered saline (PBS). Chlorhexidine (0.2%) soaked sterile cotton swabs were used for disinfecting the oral cavities of the mice, including the mandibular incisor teeth, which were immediately washed with sterile PBS. Oral streptococci were introduced to the oral cavities of all females at 4 months of age at a final concentration of $7.5 \times 10^9$ in 250 µl of PBS for 2.5 min. As an additional experiment to reproduce the early adherence of streptococci in conditions closer to a natural state, mice were given drinking water containing 1% sucrose, which is similar to the concentration in juice, 1 day before the streptococci inoculation (34). Following inoculation, samples were collected from the labial surfaces of the mandibular incisor teeth with a sterile cotton ball and then dipped in 2 ml of PBS. The samples in PBS were sonicated by ultrasonic dispersion (power output, 60 W) for 10 s, and then poured onto Mitis-Salivarius agar plates (MS) or MS containing 0.02 M bacitracin (MSB) using an EDDY JET spiral system (Gunze Sangyo, Inc., Tokyo). The number of CFU was determined after 48 h of anaerobic incubation at 37°C.

### Saliva collection and flow volume

Under anesthesia, the mice were injected with a cocktail of isoproterenol (0.20 mg/100 g body weight) and pilocarpine (0.05 mg/100 g body weight) (Sigma Chemical, St. Louis, MO) in PBS (0.1 ml) as a secretagogue. Following the intraperitoneal injection, whole saliva was collected from each mouth by a micropipette for 15 min and stored at −20°C in preweighed tubes. Volume was calculated based on the weight change of the tubes using a Mettler analytical balance.

### α-Amylase activity and protein concentration

Whole saliva samples were examined for amylase activity to hydrolyze starch using an amylase kit (Sigma Chemical). Aliquots of 20 µl of saliva diluted 1000-fold in PBS were added to 1 ml of an amylase reagent, which contained 4, 6-ethylidene (g7)-p-nitrophenyl (G1)-α, and D-maltoheptaside (ET-G7PNP), and mixed immediately by inversion then incubated at 37°C for 2 min. Absorbance was measured at 405 nm. Protein concentrations in saliva were also measured using a Bio-Rad Protein Assay (Bio-Rad Laboratory, Hercules, CA) based on the method of Bradford, an accurate procedure for determining the concentration of solubilized protein that involves the addition of an acidic dye to the protein solution and subsequent measurement at 595 nm.

### SDS, and native PAGE, and Western blot analysis

Prior to electrophoretic analysis, the saliva samples were diluted with an equal volume of SDS-PAGE sample buffer [0.06 M Tris-HCl (Amersham Pharmacia Biotech, Buckinghamshire, UK), pH 6.8, 20% glycerol (Wako Pure Chemical Industries Ltd, Osaka, Japan), 1% (wt/vol) SDS (Wako), 1% 2-mercaptoethanol (2-ME, Sigma Chemical), and 0.0012% bromphenol blue (Wako)] and native PAGE sample buffer without SDS and 2-ME. The SDS-PAGE samples were heated at 100°C for 3 min just prior to applying to the gel. The samples were then subjected to a 10% polyacrylamide gel (PAGEL-compact, ATTO Corp., Tokyo, Japan) in the presence of 0.025 M of Tris-HCl, 192 mM of glycine (Wako), 0.1% (wt/vol) SDS, and 1% 2-ME for SDS-PAGE, as well as to 5–20% gradient polyacrylamide gels (PAGEL-compact, ATTO Corp.) without SDS and 2-ME for native PAGE. Electrophoretic separation of the proteins was carried out in SDS and native PAGE for 60 min at 100 mA. For molecular weight markers, low-molecular (phosphorylase b 94.0 kDa, bovine serum albumin 66.0 kDa, ovalbumin 45.0 kDa, carbonic anhydrase 30.0 kDa, trypsin inhibitor 20.1 kDa, and α-lactalbumin 14.4 kDa) and high-molecular (thyroglobulin 669 kDa, ferritin 440 kDa, catalase 232 kDa, lactate dehydrogenase 140 kDa, and albumin 66 kDa) weight markers were

*Table 1.* Comparison of physiological and biological changes in saliva between E2F-1$^{-/-}$, C57BL/6, NOD/LtJ and 129SV mice

| Mice (*n*) | Body weight (g) | Total saliva volume (μl) | Saliva volume (μl/100 g BW) | Saliva amylase activity (IU/l) | Saliva protein concentration (μg/μl) | Saliva protein/min (μg/min) |
|---|---|---|---|---|---|---|
| E2F-1$^{-/-}$ (10) | 22.7 ± 1.6*,***,*** | 49.4 ± 17.5*,***,*** | 218.2 ± 75.3*,***,*** | 1037.4 ± 380.8* | 7.5 ± 0.8 | 23.0 ± 9.5* ** |
| C57BL/6 (10) | 25.2 ± 2.0 | 110.5 ± 22.1 | 438.2 ± 84.0 | 435.4 ± 178.3** | 6.4 ± 1.8 | 45.0 ± 13.5** |
| Nondiabetic NOD (10) | 25.9 ± 1.9 | 138.0 ± 39.2*** | 538.8 ± 160.5 | 1198.5 ± 350.0* | 7.7 ± 1.3 | 69.5 ± 5.4* |
| Diabetic NOD (6) | 25.0 ± 1.5 | 94.5 ± 65.7** | 377.7 ± 265.8 | – | – | – |
| 129SV (4) | 19.9 ± 0.7*** | 176.0 ± 20.0*** | 883.5 ± 89.9*** | – | – | – |

All values are mean ± SD.
*$P < 0.05$, vs C57BL/6 and 129SV. ** $P < 0.05$, vs nondiabetic NOD. *** $P < 0.05$, vs diabetic NOD.
–: Not detected. BW, body weight.

used (Amersham Pharmacia Biotech). When electrophoresis was completed, proteins in the gels were stained with 0.1% (wt/vol) Coomassie Brilliant Blue. For Western blot analysis, proteins in the gels were transferred to nitrocellulose membranes (ATTO Corp.), which were washed in 25 mM Tris-HCl (pH 7.4), 0.5 M NaCl, and 0.1% (v/v) Tween-20 (TTBS) buffer, and specific binding was blocked by incubating the blots for 1 h in 0.3% skim milk/TTBS. The transferred proteins were incubated for 1 h in 1/1000 rabbit polyclonal antibody mouse IgA (Nordic Immunological Laboratories, Tilburg, Netherlands) or goat polyclonal antibody mouse mucin 1 (Santa Cruz biotechnology, Santa Cruz, CA), and then washed in TTBS three times for 5 min. The membranes were then incubated for 1 h in 1/500 antirabbit and goat IgG antibody-horseradish peroxidase conjugate (Amersham Pharmacia Biotech), and washed again in TTBS three times for 5 min. Specific bindings were finally detected using ECL Plus, a Western blot detection system (Amersham Pharmacia Biotech), with a chemiluminescent reaction and a Lumishoot (ATTO Corp.) to produce the image.

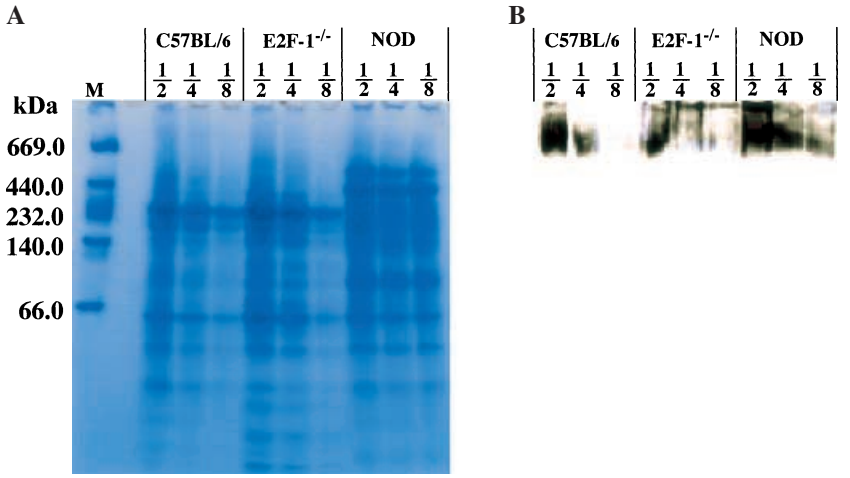


*Fig. 1.* Detection of mucin 1 in C57BL/6, E2F-1$^{-/-}$, and nondiabetic NOD mice. (A) Salivary components, as detected by Coomassie Blue staining in native PAGE. Whole saliva samples diluted by 1/2, 1/4, and 1/8 were subjected to 5–20% gradient polyacrylamide gels. M: High-molecular markers. (B) Western blot detection of mucin 1. Positive smear bands were detected in some lanes. Data are representative of three independent experiments, with similar results obtained in each.

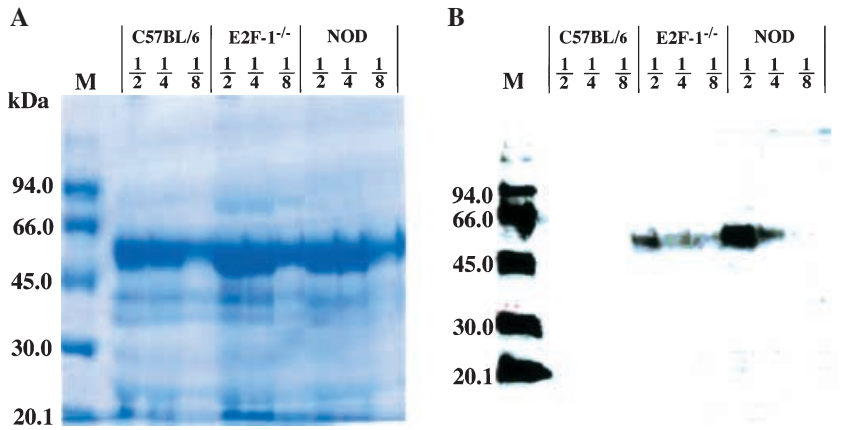


*Fig. 2.* Detection of IgA in C57BL/6, E2F-1$^{-/-}$, and nondiabetic NOD mice. (A) Salivary components, as detected by Coomassie Blue staining in SDS-PAGE. Whole saliva samples diluted by 1/2, 1/4, and 1/8 were subjected to 10% polyacrylamide gels. M: Low-molecular markers. (B) Western blot detection of IgA. Molecular weight markers are indicated on the left. Data are representative of three independent experiments, with similar results obtained in each.

### Statistical analysis

Comparative analyses were performed by ANOVA. *P*-values less than 0.01 or 0.05 were considered statistically significant for two-tailed comparisons. All statistical analyses were performed using StatView for the Macintosh operating system.

## Results

### Measurements of salivary flow and components

Table 1 summarizes the relative differences in body weight, saliva volume, amylase activity, protein concentration, and protein production/minute in E2F-1$^{-/-}$, nondiabetic and diabetic NOD/LtJ, C57BL/6, and 129SV mice. Total volume and volume per 100 g of body weight of secreted saliva were significantly smaller in E2F-1$^{-/-}$ than in the others. However, salivary amylase activity in *E2f1*-deficient mice was similar to that in nondiabetic NOD mice and higher than in C57BL/6 mice. There were no significant differences in C57BL/6, E2F-1$^{-/-}$, and nondiabetic NOD mice in salivary protein concentration. However, salivary protein production/minute in *E2f1*-deficient mice

(23.0 ± 9.5) was lower than that in nondiabetic NOD (69.5 ± 5.4), and C57BL/6 (45.0 ± 13.5) mice.

Strong mucin 1 smear expressions were observed in 1/2 dilution of whole saliva samples from all mice by Western blot analysis (Fig. 1). However, the expressions were stronger in 1/4 and 1/8 dilutions of whole saliva samples from E2F-1$^{-/-}$ and nondiabetic NOD mice than in C57BL/6 mice. Further, distinct IgA expressions were observed in 1/2, 1/4, and 1/8 dilutions of whole saliva samples from E2F-1$^{-/-}$ mice, and in 1/2 and 1/4 dilutions from NOD mice, but not in those from C57BL/6 mice (Fig. 2). Thus, amylase, mucin 1, and IgA were found in higher concentrations in the saliva of E2F-1$^{-/-}$ and NOD mice than in the control C57BL/6 mice.

### Colonization of oral streptococci on tooth surfaces

*S. mutans* adhesion results revealed that bacteria were colonized on the tooth surfaces of E2F-1$^{-/-}$ mice in higher numbers than on those in the control C57BL/6 mice at various time points (Fig. 3A). After extending the adhesion phase from 0 to 30 min, there was a decrease in biofilm growth; however, a large amount of bacteria remained on the tooth surfaces in E2F-1$^{-/-}$ mice. A significant number of bacteria (1471.5 ± 1177.6) was colonized on the tooth surfaces of E2F-1$^{-/-}$ mice at 90 min after inoculation, as compared to the control B10.D2 mice (44.0 ± 72.0) (Fig. 3A), and the colonization of *S. mutans* in E2F-1$^{-/-}$ mice was approximately 10-fold higher than that in nondiabetic NOD mice (Fig. 3B). *S. mutans, S. sanguis, S. sobrinus, S. salivarius, and S. mitis* were inoculated into the oral cavities of E2F-1$^{-/-}$ and C57BL/6 mice, after which we measured the amount of bacterial colonization on the tooth surfaces. The CFU/ml of *S. mitis* (1928.7 ± 1553.9) had a higher association than the others (less than 1200) (Fig. 4A) in E2F-1$^{-/-}$ mice, while that of each streptococci was less than 200 in C57BL/6 mice (data not shown). Furthermore, the CFU/ml of *S. mutans* (1478.1 ± 770.9) was found to be higher than that of *S. sanguis*, *S. sobrinus*, *S. salivarius*, and *S. mitis* in the E2F-1$^{-/-}$ mice that ingested 1% sucrose in water in the 24 h prior to inoculation (Fig. 4B).

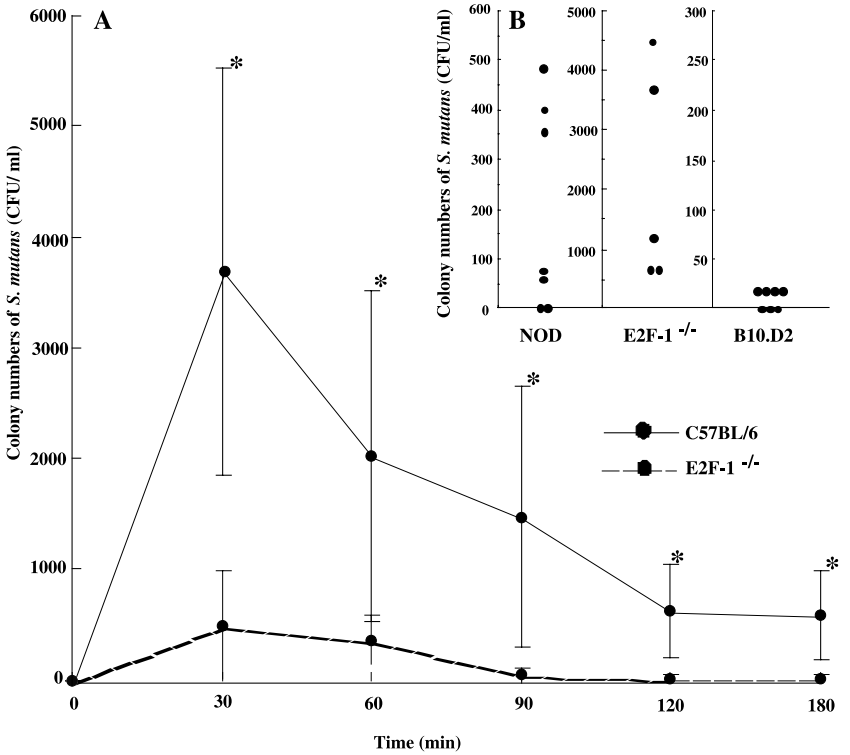


*Fig. 3.* Adhesion of *S. mutans* to tooth surfaces of experimental mice. (A) Asterisks denote significant differences (vs. C57BL/6, $*P < 0.01$). Data are representative of three independent experiments, with similar results obtained in each. (B) Adhesion of *S. mutans* in nondiabetic NOD, E2F-1$^{-/-}$, and B10.D2 female mice, 4 months of age, 90 min after inoculation. CFU were determined in MSB after 48 h of anaerobic incubation at 37°C. One dot was expressed as the number of *S. mutans* CFU per mouse. Seven NOD, five E2F-1$^{-/-}$, and seven B10.D2 mice were used for the *S. mutans* adherence assay. Data are representative of three independent experiments, with similar results obtained in each.

## Discussion

Recent studies have found that E2F-1 functions primarily as a suppressor of cell proliferation (7, 20). Along with those findings, others have observed that E2F-1 DNA binding sites in the promoters of several all-cycle genes function primarily to suppress transcription on these genes during the G0 and G1 phases (21, 40, 41), presumably by binding to the E2F-1-Rb complex. Further, some studies have suggested that suppression of transcription of the E2F-1-Rb complex suppresses entry into the S phase (7, 20). Thus, *E2f1*-deficient cells may be unable to tether Rb to E2F-1 DNA binding sites during the G0 or G1 phase, which may lead to an aberrant expression of particular cell cycle regulatory genes and inappropriate cell proliferation (20). It has been speculated that a direct role of E2F-1 as a suppressor of cell cycle progression may explain the phenotypes that some investigators have observed in *E2f1*-deficient mice, such as testicular atrophy, hyposalivation or exocrine gland dysplasia, increased mortality, hair loss, and sporadic skin tumor formation (7, 16, 20, 42).

In the present study, we examined the role of *E2f1* in susceptibility to adherence of oral streptococci in model animals with dry mouth and compared the results with NOD mice, who naturally develop Sjögren's syndrome and dry mouth. The prominent features of these diseases are manifested in the *E2f1*-deficient mice by dry mouth and hyposalivation (4, 35, 37). Further, we found a decreased production in the volume of saliva, along with increased amylase activity and IgA and mucin 1 concentrations as compared with wild type mice (C57BL/6). However, distinct differences in IgA and mucin 1 concentrations between E2F-1$^{-/-}$ and NOD mice were not observed. In addition, salivary protein production/minute in *E2f1*-deficient mice was lower than in nondiabetic NOD and C57BL/6 mice, though the differences in salivary protein concentration were not significant. Collectively, decreased levels of saliva were seen in both E2F-1$^{-/-}$ and NOD mice; however, lower levels and slower production of both saliva liquid and protein were found in E2F-1$^{-/-}$ mice than in the NOD mice. In previous histologic experiments, destruc-

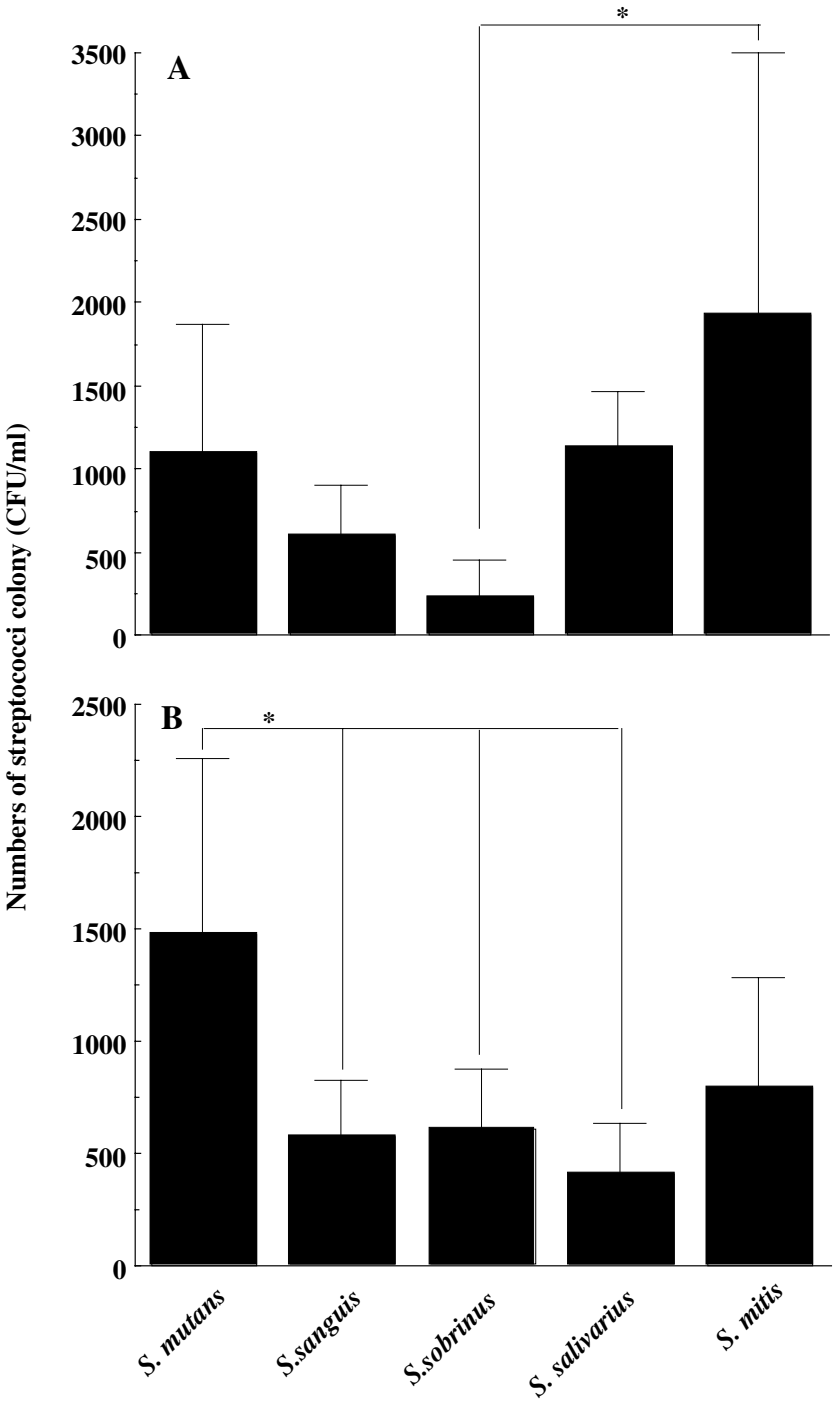


*Fig. 4.* (A) Adhesion of *S. mutans, S. sanguis, S. sobrinus, S. salivarius, and S. mitis* in E2F-1$^{-/-}$ female mice, 4 months of age, 90 min after inoculation. (B) Adhesion of the same bacteria after oral ingestion of 1% sucrose in water. CFU were determined in MS after 48 h of anaerobic incubation at 37°C. Data were obtained from three independent experiments and are expressed as the mean ± SD. *$P < 0.05$.

tion of salivary gland tissues by infiltration of lymphocytes in NOD mice was noted in one report (27), whereas in another, salivary gland section showed no infiltration of lymphocytes and large or doubled nuclei in E2F-1$^{-/-}$ mice (42). It is therefore suggested that the E2F-1$^{-/-}$ mouse has different phenotypes for decreasing saliva and dry mouth than the NOD mouse.

It has been reported that salivary components play important roles in controlling microbial colonization in the oral cavity of individuals with Sjögren's syndrome or dry mouth (37), and dry mouth may lead to qualitative and quantitative changes in the protective salivary films or pellicles that coat hard and soft tissues (3, 4). This loss of protective activities in the oral cavity could result in an increase in dental caries and gingivitis. Streptococcal adhesion is often the result of specific interactions between the carbohydrate portions of receptor glycoproteins and protein complexes, which are known as adhesins. Further, alterations in the mucosal pellicle may make oral soft tissues more susceptible to colonization by opportunistic microflora (17–19). The *S. mutans* adhesion results seen in the present experiments demonstrated that a significant number of bacteria were colonized on the tooth surfaces of E2F-1$^{-/-}$ mice, as compared to C57BL/6 and B10.D2 mice (Fig. 3A, B). In addition, in E2F-1$^{-/-}$ mice a larger number of *S. mutans* organisms adhered to tooth surfaces than in the NOD mice (Fig. 3B). NOD mice are not suitable for studies of long-term infection by oral bacteria as the average lifespan is short compared with other mice. Therefore, E2F-1$^{-/-}$ mice have benefits for *in vivo* experiments regarding oral streptococcus adhesion to tooth surfaces as compared to wild type and NOD mice. Our present results with those mice showed that the *E2F-1* gene is involved in susceptibility not only to dry mouth but also to bacterial adherence of oral streptococci to tooth surfaces.

A problem facing *in vivo* oral biofilm research is the lack of a naturalistic, reproducible, longitudinal monitoring system that permits the assessment of dry mouth and oral bacterial infection in the same animals throughout the duration of the study. Studies of *S. mutans* infection 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) (10, 38, 43). In other studies of infection, mice were provided with either a 5% sucrose or sucrose-free diet, in which the 56% of the sucrose in diet 2000 was replaced with wheat flour, and the presence of sucrose facilitated the colonization of *S. mutans*, even when smaller *S. mutans* inocula were used (12, 25). However, continuous ingestion of food containing such excess amounts of sucrose is unusual (34). Since *S. mutans* adherence to tooth surfaces may depend on a balance between physical adherence and synthesis of insoluble glucan in a natural environment, those previous methods may be inappropriate for investigation of the natural biofilm formation by streptococci, including *S. mutans* and *S. sanguis*.

In the present study, the importance of salivary flow for controlling the initial adhesion of oral streptococci in the oral cavity was demonstrated in mouse models of Sjögren's syndrome and dry mouth. Recently, we established recombinant NOD.*B10.D2* mice by replacing major histocompatibility complex (MHC) class II ($A^{g7}$, $E^{g7}$) and class $ID^{b}$ with MHC class II ($A^{d}$, $E^{d}$) and class I $D^{d}$, and found them to be nondiabetic and suitable for studies of long-term infection by oral streptococci, as the average lifespan is long compared with that of NOD mice (32). However, susceptibility to bacterial adherence to tooth surfaces in the E2F-$1^{-/-}$ mice was seen to be higher than that in NOD.*B10.D2* and NOD mice (data not shown). Therefore, we concluded that E2F-$1^{-/-}$ mice, which have a high sensitivity for initial adhesion, may be more useful than other types previously used for investigation of the initial adherence of streptococci onto tooth surfaces without synthesis of insoluble glucan. *S. mutans* colonization increased after oral ingestion of 1% sucrose and the affinities of the tested streptococci were considered to be close to those found on tooth surfaces in humans. Therefore, the E2F-$1^{-/-}$ mouse is proposed as a useful animal model, as it showed initial adherence activities of oral streptococci on the tooth surfaces in a manner similar to humans with dry mouth.

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## References

1. Almstahl A, Kroneld U, Tarkowski A, Wikstrom M. Oral microbial flora in Sjögren's syndrome. J Rheumatol 1999: **26**: 110–4.
2. Almstahl A, Wikstrom M, Kroneld U. Microflora in oral ecosystems in primary Sjögren's syndrome. J Rheumatol 2001: **28**: 1007–1013.
3. Bergdahl M, Bergdahl J. Low unstimulated salivary flow and subjective oral dryness. association with medication, anxiety, depression, and stress. J Dent Res 2000: **79**: 1652–1658.
4. Daniels TE, Fox PC. Salivary and oral components of Sjögren's syndrome. Rheum Dis Clin North Am 1992: **18**: 571–589.
5. Demuth DR, Golub EE, Malamud D. Streptococcal–host interactions. Structural and functional analysis of a *Streptococcus sanguis* receptor for a human salivary glycoprotein. J Biol Chem 1990: **265**: 7120–7126.
6. Douglas CW, Russell RR. The adsorption of human salivary components to strains of the bacterium *Streptococcus mutans*. Arch Oral Biol 1984: **29**: 751–757.
7. Field SJ, Tsai FY, Kuo F, et al. E2F-1 functions in mice to promote apoptosis and suppress proliferation. Cell 1996: **85**: 549–561.
8. Hamada S, Slade HD. Biology, immunology and cariogenicity of *Streptococcus mutans*. Microbiol Rev 1980: **44**: 331–384.
9. Helin K, Lees JA, Vidal M, Dyson N, Harlow E, Fattaey AA. cDNA encoding a pRB-binding protein with properties of the transcription factor E2F. Cell 1992: **70**: 337–350.
10. Jespersgaard C, Hajishengallis G, Huang Y, Russell MW, D. Smith DJ, Michalek SM. Protective immunity against *Streptococcus mutans* infection in mice after intranasal immunization with the glucan-binding region of *S. mutans* glucosyltransferase. Infect Immun 1999: **67**: 6543–6549.
11. Kaelin WG Jr, Krek W, Sellers WR, et al. Expression cloning of a cDNA encoding a retinoblstoma-binding protein with E2F-like properties. Cell 1992: **70**: 351–364.
12. Keyes PH, Jordan HV. Periodontal lesions in the Syrian hamster. III. Findings related to an infectious and transmissible component. Arch Oral Biol 1964: **9**: 377–400.
13. Kolenbrander PE. London J. Adhere today, here tommorow: oral bacterial adherence. J Bacteriol 1993: **175**: 3247–3252.
14. La Thangue NB. DTRF1/E2f: an extanding family of heterodimeric transcription factors implicated in cell–cycle control. Trends Biochem Sci 1994: **19**: 108–114.
15. Liljemark WF, Gibbons RJ. Proportional distribution and relative adherence of *Streptococcus miteor* (mitis) on various surfaces in the human oral cavity. Infect Immun 1972: **6**: 852–859.
16. Lillibridge CD, O'Connell BC. Human salivary gland cells, over expression of E2F1 overcomes an interferon–γ- and Tumor Necrosis Factor–α-induced growth arrest but does not result in complete mitosis. J Cell Physiol 1997: **172**: 343–350.
17. Loesche WJ. Role of *Streptococcus mutans* in human dental decay. Microbiol Rev 1986: **50**: 353–380.
18. Marsh PD. Microbiologic aspects of dental plaque and dental caries. Dent Clin North Am 1999: **43**: 599–614.
19. McEldowney S, Fletcher M. Adhesion of bacteria from mixed cell suspension to solid surfaces. Arch Microbiol 1987: **148**: 57–62.
20. Murga M. Fernandez-Capetillo O, Field SJ, et al. Mutation of E2F2 in mice causes enhanced T lymphocyte proliferation, leading to the development of autoimmunity. Immunity 2001: **15**: 959–970.
21. Neuman E, Flemington EK, Sellers WR, Kaelin WG Jr. Transcription of the *E2F-1* gene is rendered cell cycle dependent by E2F DNA-binding sites within its promoter. Mol Cell Biol 1994: **14**: 6607–6661.
22. Nevins JR. E2F: a link between the Rb tumor suppressor protein and viral oncoproteins. Science 1992: **258**: 424–429.
23. Newman F, Beeley JA, MacFarlane TW. Adherence of oral microorganisms to human parotid salivary proteins. Electrophoresis 1996: **17**: 266–270.
24. Nyvad B, Kilian M. Microbiology of the early colonization of human enamel and root surfaces *in vivo*. Scand J Dent Res 1987: **95**: 369–380.
25. Ooshima T, Sumi N, Izumitani A, Sobue S. Effect of inoculum size and frequency on the establishment of *Streptococcus mutans* in the oral cavities of experimental animals. J Dent Res 1988: **67**: 964–968.
26. Paster BJ, Boches SK, Galvin JL, et al. Bacterial diversity in human subgingival plaque. J Bacteriol 2001: **183**: 3770–3783.
27. Robinson CP, Yamachika S, Bounous DI, et al. A novel NOD-derived murine model of primary Sjögren's syndrome. Arthritis Rheum 1998: **41**: 150–156.
28. Rounbehler RJ, Rogers PM, Conti CJ, Johnson DG. Inactivation of E2f1 enhances tumorigenesis in a Myc transgenic model. Cancer Res 2002: **11**: 3276–3281.
29. Rudney JD, Hickey KL, Ji Z. Cumulative correlations of lysozyme, lactoferrin, peroxidase, S-IgA, amylase, and total protein concentrations with adherence of oral viridans streptococci to microplates coated with human saliva. J Dent Res 1999: **78**: 759–768.
30. Rudney JD, Ji Z, Larson CJ, Liljemark WF, Hickey KL. Saliva protein binding to layers of oral streptococci *in vitro* and *in vivo*. J Dent Res 1995: **74**: 1280–1288.
31. Russell MW, Mansson-Rahemtulla B. Interaction between surface protein antigens of *Streptococcus mutans* and human salivary components. Oral Microbiol Immunol 1989: **4**: 106–111.
32. Salam MA, Matsumoto N, Matin K, et al. Establishment of animal model for initial adhesion of oral streptococci using recombinant NOD.*B10.D2* mice. Clin Diagn Lab Immunol 2004: **11**: 379–386.
33. Senpuku H, Kato H, Todoroki M, Hanada N, Nisizawa T. Interaction of lysozyme with a surface protein antigen of *Streptococcus mutans*. FEMS Microbiol Lett 1996: **139**: 195–201.
34. Senpuku H, Matin K, Abdus SM, et al. Inhibitory effects of MoAbs against a surface protein antigen in real-time adherence *in vitro* and recolonization *in vivo* of *Streptococcus mutans*. Scand J Immunol 2001: **54**: 109–116.
35. Serreze DV, Chapman HD, Varnum DS, et al. B lymphocytes are essential for the initiation of T cell-mediated autoimmune diabetes: analysis of a new speed congenic stock of NOD. $Ig^{null}$ mice. J Exp Med 1996: **184**: 2049–2053.
36. Shan B, Lee WH. Deregulated expression of E2F-1 induces S-phase entry and leads to apoptosis. Mol Cel Biol 1994: **14**: 8166–8173.
37. Sreebny LM, Zhu WX. The use of whole saliva in the differential diagnosis of Sjögren's syndrome. Adv Dent Res 1996: **10**: 17–24.
38. Suzuki N, Kurihara Y, Kurihara Y. Dental caries susceptibility in mice is closely linked to the H-2 region on chromosome 17. Caries Res 1998: **32**: 262–265.
39. Van Houte J, Gibbons RJ, Banghart SB. Adherence as a determinant of the presence

of *Streptococcus salivarius* and *Streptococcus sanguis* on the human tooth surface. Arch Oral Biol 1970: **15**: 1025–1034.
40. Weintraub SJ, Chow KN, Luo RX, Zhang SH, He S, Dean DC. Mechanism of active transcriptional repression by the retinoblastoma protein. Nature 1995: **375**: 812–815.
41. Weintraub SJ, Prater CA, Dean DC. Retinoblastoma protein switches the E2F site from positive to negative element. Nature 1992: **358**: 259–261.
42. Yamasaki L, Jacks T, Bronson R, Goillot E, Harlow E, Dyson NJ. Tumor induction and tissue atrophy in mice lacking E2F-1. Cell 1996: **85**: 537–548.
43. Zhang P, Jespersgaard C, Lamberty-Mallory L, et al. Enhanced immunogenicity of a genetic chimeric protein consisting of two virulence antigens of *Streptococcus mutans* and protection against infection. Infect Immun 2002: **70**: 6779–6787.
44. Zhu JW, DeRyckere D, Li FX, Wan YY, DeGregori J. A role for E2F1 in the induction of ARF, p53, and apoptosis during thymic negative selection. Cell Growth Differ 1999: **10**: 829–838.