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Colonization of the Murine Oral Cavity by *Streptococcus gordonii*

DIANE M. LOACH,[1] HOWARD F. JENKINSON,[2] AND GERALD W. TANNOCK[1]*

Department of Microbiology[1] and Department of Oral Biology and Oral Pathology,[2] University of Otago, Dunedin, New Zealand

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***Streptococcus gordonii* DL1 (Challis) colonized the oral cavities of BALB/c mice that lacked streptococci, enterococci, and lactobacilli (LF mice) as members of an otherwise complex digestive tract microflora. Conventional mice, in comparison, were refractory to colonization by *S. gordonii*. Mice that harbored lactobacilli but were free of streptococci and enterococci (EF mice) had a lower incidence of colonization by *S. gordonii* than LF animals. The LF mouse system should be useful in the study of the molecular mechanisms that enable *S. gordonii* to inhabit the oral cavity.**

Members of the species *Streptococcus gordonii* are among the earliest colonizers of the tooth surfaces of humans and are numerous in coronal plaque, as well as in gingival crevices and on buccal and pharyngeal mucosal surfaces (5). They can also be detected in abscesses and are etiological agents in bacterial endocarditis (7). At all sites of colonization or infection, adherence is a prerequisite for streptococcal proliferation. In vitro adherence assays have demonstrated that *S. gordonii* has diverse adherence abilities since these bacteria adhere to surfaces coated with salivary components, such as mucins (13), proline-rich proteins (6), agglutinin (9), and fibronectin (14), and bind α-amylase (4, 18), immunoglobulin A (8), and serum agglutinin (8). In addition, the streptococcal cells coaggregate with a variety of other oral bacteria such as the genera *Actinomyces*, *Fusobacterium*, and *Capnocytophaga* (11). These adherence and aggregation phenomena involve surface adhesins on streptococcal cells, but little progress has been made in defining which adhesins, detected in vitro, are significant in colonization of the oral cavity. This is largely because a suitable experimental animal system with which to study the colonization attributes of strains of *S. gordonii* has not been available. Studies of oral streptococcal colonization have concentrated on the mutant streptococcus group and have utilized germfree or specific-pathogen-free rodents fed a high-sucrose, cariogenic diet to promote the production of bacterial extracellular polysaccharide and hence glucan-mediated adherence to oral surfaces (1, 3, 25). We describe here the colonization of the murine oral cavity by *S. gordonii* DL1 (Challis). These studies form a basis for future investigations in which isogenic strains of streptococci, differing only in the profile of surface proteins that they synthesize, will be compared in terms of their ability to colonize the oral cavity of an animal fed a standard (i.e., not high-sucrose) diet. *S. gordonii* DL1 (Challis) is of human origin (endocarditis) (16) and, genetically, is the best characterized strain of the species. It has cell surface-associated molecules and in vitro adhesive properties similar to those of strains M5 (17) and PK488 (12), which were isolated from the oral cavities of humans.

During studies of the digestive tract microflora of mice, we derived two colonies of BALB/c mice that were maintained in isolators by gnotobiotic methods (15, 21). The progenitors of the colonies were conventional mice, so a complex microflora was present in the animals of both colonies. Mice in one colony, unlike conventional animals, did not harbor *Streptococcus* or *Enterococcus* species as part of their normal microflora. These *Enterococcus*-free mice (EF mice) had a gastrointestinal microflora functionally equivalent to that of conventional mice on the basis of observations concerning 26 microflora-associated characteristics (21, 22). Mice belonging to the other colony did not harbor streptococci, enterococci, or lactobacilli (LF mice). They also lacked some (undetermined) members of the cecal microflora as evidenced by an enlarged cecum (15). Because of the absence of gram-positive cocci and lactobacilli in the microflora of each of these two colonies of animals, we wished to investigate the mice for their potential in studying the colonization of the oral cavity by *S. gordonii*. Conventional mice are refractory to colonization by these bacteria, possibly because of the general phenomenon of microbial interference (20).

The methods used in the maintenance of the animal colonies have been described previously (22), but it is important to note here that the sterile, rodent diet (pellets B composed of maize meal, skim milk powder, meat meal, pollard, barley, blood, lucerne, linseed, molasses, salt, and vitamin premix 202; Central Feeds, Levin, New Zealand) (2) fed to the animals was not supplemented with sucrose. As a result of preliminary experiments, the following inoculation protocol was followed in testing the colonization ability of *S. gordonii* DL1. The experimental animals (6-week-old males and females), in an isolator, were lightly anesthetized with ether and inoculated per os with 100 μl of a 24-h tryptic soy broth (Difco Laboratories, Detroit, Mich.) culture of the streptococcus. Ten milliliters of culture was added to 100 ml of the animals' drinking water on that day and the following 3 days. Sterile water in a fresh bottle was supplied to the animals thereafter. In some experiments, two or three mice were removed from the isolator and examined at 1, 4, and 7 days after final exposure to drinking water containing streptococci. In other cases, all of the animals in an experiment were examined at 7 days after final exposure. Animals were removed from the isolator, anesthetized with ether, and administered 40 μg of pilocarpine (Sigma Chemical Co., St. Louis, Mo.) subcutaneously to stimulate saliva flow. Saliva was collected from each mouse, after which the animals were killed by carbon dioxide anesthesia and cervical dislocation. The oral cavities of the mice were then sampled with sterile cotton swabs rubbed over all oral surfaces. In one experiment, the sites of *S. gordonii* colonization within the oral cavities were detected with small cotton swabs after the upper and lower jaws of each mouse had been separated by dissec-

* Corresponding author. Mailing address: Department of Microbiology, University of Otago, P.O. Box 56, 700 Cumberland Street, Dunedin, New Zealand. Fax: 3-479-8540.

TABLE 1. Detection (culture of oral swab) of *S. gordonii* in the oral cavities of mice

Murine group	Expt no.	No. of mice harboring *S. gordonii*/no. examined on day[a]:		
		1	4	7
Conventional	1	0/2	0/2	0/2
	2	0/2	0/2	0/2
EF	1	0/2	2/2	1/3
	2	1/2	0/2	1/2
LF	1	2/2	2/2	3/3
	2	2/2	2/2	2/3

[a] Day(s) after last *S. gordonii* inoculum.

tion. Two microliters of saliva was used to inoculate (by spreading) a blood agar plate (5% defibrinated horse blood in tryptic soy agar) containing 35 μg of kanamycin per ml of medium, and another 2 μl was used to inoculate a lactobacillus MRS agar (Difco) plate. Other kanamycin-blood agar plates and MRS agar plates were inoculated with the oral swabs. Kanamycin was added to the blood agar in order to suppress the growth of gram-negative facultatively anaerobic bacilli which otherwise prevented detection of *S. gordonii* (resistant to this concentration of kanamycin). The plates were incubated at 37°C, under microaerophilic conditions, for 48 h.

Comparison of the oral microflora of the mice was made by culturing saliva samples and oral swabs of mice that had not been inoculated with *S. gordonii*. The following media were used: blood agar (aerobic, microaerophilic, or anaerobic [glovebox] incubation; nonselective), blood agar-kanamycin (microaerophilic; streptococci), MRS agar (microaerophilic; gram-positive cocci and bacilli), Tergitol-7 agar (Difco) (aerobic; coliforms), CFAT agar (anaerobic; actinomyces) (26), mitis-salivarius agar (Difco) (microaerophilic; streptococci), and medium 10A agar (anaerobic; lactobacilli) (19). All incubations were at 37°C for 48 h, except that of Tergitol-7 agar, which was for 24 h.

Results comparing the colonization of the oral cavities of conventional (housed in an open animal room), EF, and LF mice by *S. gordonii* are given in Tables 1 and 2. Streptococci did not colonize the oral cavities of conventional mice but were detected in samples collected from about 54% of EF mice examined 7 days after exposure to water containing *S. gordonii*. Colonization of the oral cavities of LF mice was observed in approximately 89% of the animals examined at 7 days, and streptococci could be enumerated in saliva samples (Table 2). Swabbing specific oral surfaces of LF mice showed that *S. gordonii* was present on palate, tongue, and tooth surfaces as is the case in human subjects (5).

TABLE 2. Detection of *S. gordonii* in mice 7 days after last inoculum

Murine group	Expt no.	No. of mice harboring *S. gordonii*/ no. examined[a]	Log_{10} median of CFU/ml of saliva (range)
Conventional	1	0/7	ND[b]
EF	1	6/11	ND (ND–3.2)
LF	1	9/10	2.8 (ND–3.3)
	2	8/9	2.7 (ND–3.8)
LF + lactobacilli	1	6/9	ND (ND–3.2)
LF + streptococci	1	0/9	ND

[a] Examination by oral swab.
[b] ND, none detected, i.e., <2.7 CFU/ml.

TABLE 3. Qualitative comparison of oral microfloras (cultures of swabs) of murine groups

Murine group	No. of mice harboring the specified bacteria/no. examined				
	Lacto-bacilli	Coryne-bacteria	Gram-negative bacilli[a]	*E. coli*	Strepto-cocci
Conventional	2/10	10/10	10/10	0/10	10/10
EF	3/4	4/4	4/4	1/4	0/4
LF	0/6	6/6	6/6	4/6	0/6

[a] Other than *E. coli*.

Comparison of the oral microflora of mice originating in the different murine colonies showed that corynebacteria (gram-positive bacilli with coryneform morphology and biochemical test results appropriate to the genus) (10) and gram-negative facultatively anaerobic bacilli (*Flavobacterium* and *Moraxella*-like species according to 20E strips [API System, La Balme les Grottes, France]) were common to all groups (Tables 3 and 4). Lactobacilli were absent from the microflora of LF animals but present in those of EF and conventional mice. Streptococci were detected only in samples from conventional mice. *Escherichia coli* (identified with API 20E strips) was detected in LF and EF samples but not in those collected from conventional mice (Table 3). The absence of detectable numbers of obligate anaerobes in the murine oral cavity is noteworthy, as has been observed by others (24). Examination of Gram-stained smears of saliva, tooth scrapings, and material obtained by swabbing mucosal surfaces confirmed the relative simplicity of the murine oral microflora, with gram-negative bacilli, gram-positive coryneforms, and gram-positive cocci being the morphotypes seen in samples from conventional mice.

In order to investigate the reason for the lack of *S. gordonii* colonization in conventional mice, and the lower incidence of colonization in EF than in LF animals, we conducted the following experiments. LF mice in one group were inoculated with the three strains of lactobacilli that were present in EF animals (*Lactobacillus delbrueckii* 18 and 21 and *Lactobacillus fermentum* 20) (23). Another group of LF mice in a separate isolator was inoculated with the three streptococcal strains detected in conventional mice (two alpha-hemolytic and one nonhemolytic strain, each with a different colony morphology and biochemical profile in API 20 STREP strips). One week after inoculation with these bacteria, both groups of mice were inoculated with *S. gordonii* as described above. The mice were examined 7 days after their last exposure to *S. gordonii*. The strains of lactobacilli and streptococci used as initial inocula colonized the mice prior to inoculation of the animals with *S. gordonii* as evidenced by the recovery of these strains from oral swabs (streptococci) and feces (lactobacilli) and subsequent morphological and biochemical characterization. The presence of lactobacilli in the murine microflora reduced the incidence of colonization by *S. gordonii* in the oral cavities of ex-LF mice (66%) as well as lowering the number of streptococci in saliva in comparison to that of LF animals (Table 2). Streptococci originating from the oral cavities of conventional mice prevented the establishment of *S. gordonii* in the oral cavities of ex-LF mice (Table 2). We conclude that lactobacilli and streptococci indigenous to the mouse digestive tract produce major interference phenomena that normally prevent the establishment of *S. gordonii*. The apparent failure of other streptococcal species and lactobacilli to exclude *S. gordonii*

TABLE 4. Qualitative comparison of oral microfloras of murine groups

Murine group	*n*	Log_{10} median of CFU/ml of saliva (range)				
		Lactobacilli	Corynebacteria	Gram-negative bacilli[a]	*E. coli*	Streptococci
Conventional	10	ND[b] (ND–3.4)	4.4 (ND–5.5)	4.8 (4.2–5.3)	ND	4.7 (3.7–5.4)
EF	7	ND (ND–2.7)	3.2 (ND–4.2)	4.5 (4.1–5.2)	ND	ND
LF	5	ND	3.2 (2.7–3.8)	4.5 (4.2–4.7)	ND (ND–2.7)	ND

[a] Other than *E. coli*.
[b] ND, none detected, i.e., <2.7 CFU/ml.

from the human oral cavity may relate to the order in which the ecosystem is colonized by microbial strains during acquisition of the normal microflora or to subtle physiological differences that possibly exist between strains constituting the respective normal microfloras of humans and mice.

The LF mouse colony appears to provide a useful system with which to study the colonization of the oral cavity by *S. gordonii*. The mice are housed in an environment which can be maintained microbiologically constant so that individual isogenic strains of *S. gordonii* can be monitored in association with a mammalian host. Unlike germfree animals, the mice in the LF colony harbor a complex digestive tract microflora so that they have most of the microflora-associated characteristics of conventional mice (15, 22). The mice provide the potential to investigate not only microbe-host interactions but also microbial coaggregation phenomena. A further advantage of this experimental animal system is that a cariogenic diet, rich in sucrose, is not necessary to achieve colonization of the oral cavity by *S. gordonii*.

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