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Suppression of *Candida albicans* by Human Oral Streptococci in Gnotobiotic Mice

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Mixed human salivary bacteria and strains of *Streptococcus salivarius* and *S. miteor* suppressed colonization of *Candida albicans* in gnotobiotic mice. *C. albicans* attached in lower numbers to epithelial cells from conventional rats than from germ-free rats, and attachment inhibition by indigenous flora may explain in part the suppression of *Candida* colonization.

Candida albicans commonly colonizes the oral cavity and intestinal canal of man and various animals (18). There are data which suggest that the indigenous bacterial flora may suppress colonization of this organism. For example, antibiotic therapy, which affects the bacterial flora, increased the frequency of overgrowth and infection by *Candida* species (16). *C. albicans* also appears to colonize germ-free chicks and mice in higher numbers than conventional animals (1, 13). Although gnotobiotic chicks possessing a monoflora of *Escherichia coli* were found to be more resistant to crop infections by *C. albicans* (2), there are few data available concerning the types of bacteria which are capable of suppressing colonization of *C. albicans* or of the mechanisms involved in this competitive microbial interaction. The present report describes the suppression of *C. albicans* by human salivary bacteria and by pure cultures of human oral strains of *Streptococcus salivarius* and *S. miteor* (*S. mitis*). In addition, a novel mechanism for the suppression of *Candida* by indigenous bacteria is proposed.

In the first series of experiments, two groups of germ-free mice (Charles River Breeding Lab.) were inoculated intraorally from a sealed ampoule containing 2 ml of a 36-h broth culture of approximately 5.0×10^7 *C. albicans* per ml which had been taken into the isolators through a peracetic acid-sterilized entry lock. Intraoral inoculation was accomplished by swabbing the oral cavity of each mouse and distributing the remainder in the diet of the animal. A third group of five animals was inoculated identically as the first two groups with a 2-ml sample of whole human saliva containing the mixed indigenous microbial population of the donor of approximately 10^8 bacteria per ml. A 2-week period after inoculation was chosen to allow the organisms introduced into the animals to stabilize. It is known from previous germ-free animal experiments that after 1 week the numbers of bacteria in the gastrointestinal tract and oral cavity reach a maximum level (Gibbons, unpublished data). Thus, 2 weeks after infection, during which the establishment of the yeast and salivary organisms was verified, a second sample of whole human saliva was introduced into the mouths of one group of *Candida*-infected mice, and a pure culture of *C. albicans* was introduced into the mouths of animals initially contaminated with salivary organisms. The animals were sacrificed 2 weeks after the secondary inoculation, and the numbers of *C. albicans* per gram of fecal contents was determined for each animal by using Trypticase soy agar (BBL) supplemented with 7.5 μg of vancomycin and 200 μg of streptomycin/ml. This medium yielded colony counts from *C. albicans* suspensions which were comparable to blood agar, but it inhibited the growth of most indigenous bacteria. To determine the number of *C. albicans* in the oral cavity, the skin was removed from the head of each animal to eliminate contamination. Each head was homogenized (Sorvall omnimixer) in buffered saline, and the total number of *C. albicans* per head was determined.

It was found that *C. albicans* attained a high population density in both the oral cavity and intestinal canal of monoinfected animals (Table 1). Similar or lower population levels of *Candida* were attained when this organism was introduced into the mouths of mice which had been previously colonized by salivary bacteria.

When samples of homogenized head suspen-

sions of animals infected with salivary organisms were cultured on Mitis-Salivarius agar (Difco), the most prominent streptococci observed were *S. salivarius* and *S. miteor*. These *Streptococcus* species are known to colonize human oral epithelium, and they are found in highest proportions on the tongue and cheek surfaces, respectively (11, 17). Consequently, strains of each species were tested individually to determine their ability to suppress *C. albicans*. For comparison, a strain of *S. mutans* was also included since it is known that in humans this organism preferentially colonizes teeth and is present in only low proportions on oral epithelial surfaces (6, 17). Groups of five germ-free mice were monoinfected with *C. albicans*, and 2 weeks later the animals were secondarily infected with either *S. salivarius* strain 9GS2, *S. miteor* strain 26, or *S. mutans* strain 6715. The numbers of *C. albicans* in the oral cavity and in the feces of the animals was determined 2 weeks after the secondary infection. The population levels of each *Streptococcus* species were also determined by using Mitis-Salivarius agar.

It was found that pure cultures of both *S. salivarius* and *S. miteor* were capable of suppressing *C. albicans* in the intestinal canal and in the oral cavity of the mice to an extent which was comparable to the mixed salivary flora (Table 2). However, *S. mutans* strain 6715 did not affect the *Candida* populations in feces or in the oral cavity, even though this streptococcus colonized the animals in high numbers (Table 2).

Previous investigators have suggested that the indigenous bacterial flora may suppress growth of *C. albicans* by either secreting antifungal substances or by competing for nutrients (8, 9, 12, 15, 16). However, no zones of inhibition indicative of the production of growth-inhibiting materials were observed when cultures of *S. salivarius* and *S. miteor* were cross-streaked with *C. albicans* on Trypticase soy blood agar (BBL). In addition, *S. salivarius*, which effectively suppressed *C. albicans*, is generally considered to have similar nutritional requirements as *S. mutans* (3, 4, 14). Therefore, the observation that *S. mutans* did not reduce the *Candida* populations of the mice, even though this streptococcus colonized comparably to the other streptococci studied, does not support nutrient competition as being the primary factor involved in the suppression of *Candida*.

The colonization of mucosal surfaces has recently been shown to be dependent upon factors in addition to those which affect microbial growth. For example, differential growth rates are commonly considered as a factor which may contribute to the numerical predominance of certain microorganisms over others. However, this may be of secondary importance in the oral cavity because organisms found there must first attach to various surfaces to resist quantitative removal by bathing secretions (5–7, 10, 11, 17). Indigenous and pathogenic bacteria have been found to differ widely in their abilities to attach to mucosal and other surfaces, and the ability of an organism to adhere to a surface has been

TABLE 1. *Effect of human salivary bacteria on the colonization of C. albicans in germ-free mice*

Initial inoculum of mice	Secondary inoculum of mice	Mean no. of *C. albicans* per:	
		Oral cavity	Gram feces
C. albicans		$5.5 \pm 1.1 \times 10^{3}$ [a]	$1.1 \pm 0.62 \times 10^{8}$ [a]
C. albicans	Mixed salivary flora	$6.6 \pm 2.1 \times 10^{2}$	$1.8 \pm 0.51 \times 10^{6}$
Mixed salivary flora	*C. albicans*	$4.0 \pm 2.2 \times 10^{2}$	$4.5 \pm 2.3 \times 10^{5}$

[a] Mean and standard errors of mean from five mice.

TABLE 2. *Ability of strains of S. salivarius, S. miteor, and S. mutans to suppress colonization of C. albicans in gnotobiotic mice*

Secondary inoculum of mice	No. of *C. albicans* per:		No. of *Streptococci* per:	
	Oral cavity	Gram feces	Oral cavity	Gram feces
None	$6.3 \pm 3.1 \times 10^{3}$ [a]	$1.4 \pm 0.34 \times 10^{8}$ [a]		
S. salivarius strain 9GS2	$2.2 \pm 0.73 \times 10^{2}$	$1.6 \pm 0.93 \times 10^{6}$	$2.2 \pm 1.0 \times 10^{4}$ [a]	$9.9 \pm 4.2 \times 10^{8}$ [a]
S. miteor strain 26	$3.1 \pm 0.58 \times 10^{2}$	$6.4 \pm 4.9 \times 10^{6}$	$9.8 \pm 5.2 \times 10^{3}$	4.4×10^{8} [b]
S. mutans strain 6715	$7.1 \pm 1.2 \times 10^{3}$	$1.2 \pm 0.25 \times 10^{8}$	$7.4 \pm 5.1 \times 10^{3}$	$3.9 \pm 0.32 \times 10^{8}$

[a] Mean and standard error of mean from five mice.
[b] Count from only one animal.

found to correlate with the extent to which it colonized indigenously. Thus, it is possible that the indigenous bacterial flora could suppress the extent of colonization of *C. albicans* by interfering with its ability to attach to mucosal surfaces. Consequently, the ability of cells of *C. albicans* to attach to tongue and cheek epithelial cells of germ-free and conventional rats was determined. A previously described in vitro procedure (7) was employed in which 2.0×10^8 yeast cells were incubated with 10^5 epithelial cells for 60 min. It was found that twice as many cells of *C. albicans* became attached to tongue and cheek epithelial cells from germ-free rats as compared to cells from conventional animals (Table 3). These differences in adherence were statistically significant ($P < 0.001$). This observation compares positively with the in vivo experiments previously described in Tables 1 and 2. The in vivo experiments clearly show that *C. albicans* establishes in much higher numbers in germ-free mice than mice which have been previously exposed to other microorganisms. Similarly, in the in vitro experiments those cells obtained from conventional rats have been exposed to other microorganisms. Thus, it appears that the presence of an indigenous bacterial flora can interfere with the adherence of *C. albicans* to epithelial surfaces. This could be achieved by competing for, or otherwise modifying, epithelial receptor sites required for *Candida* attachment or by enzymatically altering the surface of the yeast cells. Since mucosal surfaces continuously desquamate, organisms on these surfaces, or their progeny, must constantly reattach for colonization to continue. Over a period of time, this would be expected to greatly magnify the ecological effects of the twofold difference in adherence observed. The observations presented in this communication support the hypothesis that the bacterial suppression of *C. albicans* populations on mucosal surfaces may be mediated in part by an interference with the adherent interactions of this yeast. Such a mechanism helps to explain why *S. salivarius* and *S. miteor*, which have been shown to adhere well to oral mucosal surfaces (7, 11, 17), were able to suppress *C. albicans* in the oral cavity, whereas *S. mutans*, which is known to attach poorly to oral epithelial cells (7, 11), had little effect. Unfortunately, there is little information available concerning the adherence of these streptococci to intestinal mucosa. The ecological principle proposed seems likely to be applicable to other competitive microbial interactions which are recognized to occur on the mucosal surfaces of man.

TABLE 3. *Adherence of C. albicans to germ-free and conventional rat tongue and cheek epithelial cells*

Source of epithelial cells	No. of *C. albicans* epithelial cells[a]	
Germ-free tongue	8.1 ± 0.64[a]	
		$P = < 0.001$[b]
Conventional tongue ..	3.9 ± 0.38	
Germ-free cheek	9.1 ± 0.66	
		$P = < 0.001$
Conventional cheek ...	5.2 ± 0.42	

[a] Mean and standard error of mean derived from three separate experiments involving counts of 150 epithelial cells.

[b] Probability derived from *t* test.

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