INFECTION AND IMMUNITY, Nov. 1990, p. 3813–3815
0019-9567/90/113813-03$02.00/0
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Vol. 58, No. 11

Resistance of Congenitally Immunodeficient Gnotobiotic Mice to Vaginal Candidiasis

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Congenitally immunodeficient beige, athymic, and beige athymic mice whose orogastric mucosal tissues were chronically colonized and infected with a pure culture of *Candida albicans* were found to be resistant to naturally occurring vulvovaginal candidiasis.

Candida albicans is often a member of the normal flora of the female genital tract (23). The conversion of *C. albicans* from a commensal to a pathogen is associated with a number of predisposing factors including impaired cellular immunity, pregnancy, diabetes, antibiotics or steroid therapy, oral contraceptives, and iron deficiency anemia (21, 24). *C. albicans* is also a common cause of recurrent vaginitis among otherwise apparently healthy women of child-bearing age (11, 13, 18, 24). Oral candidiasis has been identified as an indicator of severe immunodeficiency and as an early predictor of infection with the human immunodeficiency virus (15). Approximately 24% of human immunodeficiency virus-infected women have a history of chronic refractory vaginal candidiasis. In addition to vaginal candidiasis, all of these acquired immunodeficiency syndrome patients were found to have oral thrush (15). Although vaginal candidiasis is treatable, therapy is often ineffective and recurrence is common (18, 24).

Rodent models of experimental vaginal candidiasis have been used to study the efficacy of antifungal agents (10, 17, 20). Chronic infection of these animals is dependent on the maintenance of pseudoestrous either by oophorectomy or the use of hormones (10, 20, 22). Hormonal disturbances either following oophorectomy or estrogen treatment have a number of negative effects on the health and immune status of the host (3, 4, 9, 14). Congenitally immunodeficient mice could be used in the absence of hormone-altering procedures to mimic and pinpoint immunodeficiencies that predispose to vulvovaginal candidiasis (11, 19, 21). Congenitally immunodeficient inbred mice with defects in cell-mediated immunity (nude [*nu/nu*] mice) or innate immunity (beige [*bg/bg*] mice) and combined defects in innate immunity and cell-mediated immunity (beige nude [*bg/bg nu/nu*] mice) are available and have been well characterized (8, 16, 19, 25). We have previously shown that mucosal surfaces of the alimentary tract of congenitally immunodeficient germfree mice are quickly and chronically colonized and infected with *C. albicans* (1, 5). Because of reports which show concomitant oral and vaginal *C. albicans* infections in immunocompromised patients (15), we carried out these studies to determine whether the mucosal surfaces of the reproductive tract in congenitally immunodeficient mice are also naturally susceptible to colonization and infection by *C. albicans*.

Congenitally immunodeficient mice were produced by mating homozygous (*nu/nu*, *bg/bg*, and *bg/bg nu/nu*) males with heterozygous (*nu/+*, *bg/+*, and *bg/bg* nu/+, respectively) females. Germfree mice were originally derived from NIH BALB/c nude mice, NIH C57BL/6 beige mice, and N:NIH(S)III beige nude mice by cesarean derivation and have since been bred and housed in flexible film isolators at the University of Wisconsin Gnotobiotic Research Laboratory (Madison, Wis.). The microbial status of mice was assessed by methods previously described (1).

The gastrointestinal tracts (GI) of 5- to 8-week-old germfree female mice were colonized with a pure culture of *C. albicans* as previously described (5). For some experiments, mice were also inoculated intravaginally, using a 20-gauge feeding needle, with 10^6 *C. albicans* per mouse. Mice were sacrificed in groups of three, the ovaries, uterus, and vagina were homogenized, and dilutions were cultured on Sabouraud dextrose agar. Prior to sacrifice, vaginal smears of mice were taken and stained with Diff-Quik stain (American Scientific Products, McGaw Park, Ill.) in order to determine the stage of the estrous cycle of each mouse (22). Additional mice were sacrificed, and their tissues were embedded in plastic, sectioned (2 μm), and stained with periodic-acid Schiff followed by either azure A-eosin B or hematoxylin and eosin stain. Stained sections were viewed with a light microscope.

A common source of *C. albicans*, which may cause vaginitis in women, is the patients' own GI tract microflora (6). Gnotobiotic female mice whose GI tracts were chronically colonized with high numbers of a pure culture of *C. albicans* for 1 to 12 weeks were sacrificed, and the genital tracts were cultured for *C. albicans*. Immunocompetent (*nu/+* and *bg/+*) and singly immunodeficient (*nu/nu* and *bg/bg*) mice whose GI tracts were heavily colonized with *C. albicans* (6 to 8 $\log_{10}$ CFU/g [dry weight]) showed from 0 to 4.7 $\log_{10}$ CFU/g (dry weight) of *C. albicans* in the vagina at 2 weeks after *C. albicans* GI tract colonization (data not shown). Very low numbers of *C. albicans* were sporadically cultured from the ovaries and uterus of these same mice. The numbers of *C. albicans* cultured from the vaginas of *nu/+*, *bg/+*, *nu/nu*, or *bg/bg* mice remained low or decreased after 2 weeks of GI tract colonization regardless of which stage of the estrous cycle that the mice were in at the time of culturing. In contrast to the athymic or beige mouse strains, *C. albicans* was consistently cultured from the vaginas of doubly immunodeficient *bg/bg nu/nu* or singly immunodeficient *bg/bg nu/+* mice for up to 12 weeks after GI tract colonization (data not shown). The highest number of *C.*

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TABLE 1. Correlation of estrous cycle with the isolation of *C. albicans* from the vaginas of *bg/bg nu/nu* and *bg/bg nu/+* mice[a]

Stage in estrous cycle	Log_{10} CFU/organ in mice (mean ± SEM)	
	bg/bg nu/nu	*bg/bg nu/+* (*n*)
Proestrus	0.2 ± 0.1 (5)	0 (5)
Estrus	2.5 ± 0.1 (2)	3.2 ± 0.5 (5)
Metestrus	1.2 ± 0.2 (8)	2.7 ± 0.5 (3)
Diestrus	0.3 ± 0.3 (3)	0.2 ± 0.1 (5)

[a] The GI tracts of these gnotobiotic mice were colonized with a pure culture of *C. albicans* for 1 to 12 weeks.

TABLE 3. Resistance of congenitally immunodeficient mice to naturally occurring vaginal candidiasis

Genotype of mice	No. of mice	Length of time (wks) of GI tract colonization	No. of mice positive by histology/total no.	No. of mice culture positive/total no.
bg/bg	24	1–28	0/6	10/18
bg/+	24	1–28	0/6	8/18
nu/nu	29	1–48	0/8	12/21
nu/+	29	1–48	0/8	15/21
bg/bg nu/nu	30	1–14	0/12	12/18
bg/bg nu/+	28	1–16	0/10	11/18

albicans was isolated from the vaginas of *bg/bg nu/nu* and *bg/bg nu/+* mice during estrus or metestrus (Table 1).

To ensure that equal numbers of *C. albicans* reached the genital tract of each mouse, *nu/nu*, *nu/+*, *bg/bg nu/nu*, and *bg/bg nu/+* mice were intravaginally inoculated with 10^6 *C. albicans*. Table 2 shows that the largest numbers of *C. albicans* were isolated from the vaginas of *nu/+*, *bg/bg nu/nu*, and *bg/bg nu/+* mice 1 day after inoculation. *nu/nu* mouse vaginas had their highest counts 7 days following inoculation, and these mice also appeared to have viable *C. albicans* in their ovaries and uterus (Table 2). In all cases, the colony-forming units per organ decreased over time and appeared to be completely cleared from *nu/nu*, *nu/+*, and *bg/bg nu/+* mice by day 14 regardless of which stage in the estrous cycle the mice were in at the time of culturing (Table 2). In contrast, *bg/bg nu/nu* mouse vaginas appeared to be persistently colonized with low numbers of *C. albicans* for 21 days (Table 2).

Histology of the vaginas, from 50 mice whose alimentary tract were colonized with 6 to 8 log_{10} *C. albicans*/g (dry weight) (for 2 to 48 weeks) showed no signs of infection (i.e., hyphal penetration of mucosal tissues [Table 3]). Regardless of the stage in the estrous cycle or genotype of the mouse, *C. albicans* could not be found (on histological examination) naturally infecting the mucosal surfaces of the vagina following alimentary tract colonization. *C. albicans* hyphae were observed infecting the vagina of one *nu/nu* and one *bg/bg nu/nu* mouse during late estrus or early mestestrus 14 days after intravaginal inoculation with *C. albicans* (Fig. 1). *C. albicans* infected only the keratinized portions of these vaginas, and there were no *C. albicans*-infecting areas in which the keratinized epithelial cells had already been shed.

Low numbers of *C. albicans* were found in the vaginas of mice whose GI tracts were chronically colonized with large numbers of *C. albicans*. *C. albicans* apparently reaches the vagina following GI tract colonization (Table 3) but does not appear to cause a vaginal infection in *nu/+*, *bg/+*, *nu/nu*, and *bg/bg* mice. In *bg/bg nu/nu* and *bg/bg nu/+* mice, *C. albicans* was isolated from the vagina during estrus or metestrus; however, a chronic mucosal infection was not established. Even after intravaginal inoculation with 10^6 viable *C. albicans*, high numbers of *C. albicans* could not be consistently isolated from the vaginas of *nu/nu*, *nu/+*, *bg/bg nu/+*, and *bg/bg nu/nu* mice.

As demonstrated previously, *C. albicans* has a predilection for keratinized portions of mucosal tissues in vivo (1, 2, 5, 22). This is apparently true for the vagina also. Only when the epithelial cells of the vagina are fully keratinized (estrus), have we and others observed *C. albicans* infecting the vagina (10, 20, 22). Estrus lasts an average of 1 day in a mouse (22). Following estrus, the keratinized cells are shed (possibly along with any *C. albicans* infecting them) and polymorphonuclear leukocytes appear (22). Polymorphonuclear cells are known to be very efficient inhibitors and killers of *C. albicans* (7, 12). The short duration of estrus in combination with the appearance of polymorphonuclear cells may make the murine vagina a very inhospitable place for *C. albicans*, even in congenitally immunodeficient mice.

The vaginas of doubly immunodeficient (*bg/bg nu/nu*) mice appeared to be more readily colonized with *C. albicans* than immunocompetent or singly immunodeficient (i.e., either *bg/bg* or *nu/nu*) mice. It appears that a combination of defective polymorphonuclear cells and an absence of thymus matured T cells enhances the susceptibility of the vagina to colonization with *C. albicans*. However, even doubly immunodeficient mice which are extremely susceptible to mucosal

TABLE 2. Colonization of the reproductive tract following vaginal inoculation of congenitally immunodeficient germfree mice with *C. albicans*

No. of days following inoculation[a]	Stage in estrous cycle[b]		Log_{10} CFU/organ (mean ± SEM) (*n* = 3)					
			Ovary		Uterus		Vagina	
	nu/nu	*nu/+*	*nu/nu*	*nu/+*	*nu/nu*	*nu/+*	*nu/nu*	*nu/+*
1	D, M, E	E, E, E	0.3 ± 0.3	0	0	0	0.4 ± 0.2	4.8 ± 1.0
7	E, M, M	M, E, E	3.5 ± 0.5	0	2.7 ± 1.1	0	1.4 ± 0.4	0.2 ± 0.2
14	E, P, P	D, P, P	0.9 ± 0.9	0	0.5 ± 0.5	0	0.3 ± 0.3	0
	bg/bg nu/nu	*bg/bg nu/+*	*bg/bg nu/nu*	*bg/bg nu/+*	*bg/bg nu/nu*	*bg/bg nu/+*	*bg/bg nu/nu*	*bg/bg nu/+*
1	E, E, M	E, M, E	0	0	0.4 ± 0.4	0.7 ± 0.7	2.5 ± 1.5	2.5 ± 1.3
7	M, M, M	M, E, M	0.1 ± 0.1	0	0.8 ± 0.5	0.2 ± 0.2	0.4 ± 0.3	0.1 ± 0.1
14	P, P, E	D, D, M	0	0	0.4 ± 0.4	0	1.2 ± 0.2	0.4 ± 0.4
21	D, D, M	E, E, P	0.5 ± 0.5	0	0.4 ± 0.4	0	1.5 ± 0.4	0.1 ± 0.1

[a] Mice were inoculated intravaginally with 10^6 viable *C. albicans* per mouse.
[b] The stage in the estrous cycle of each of the three mice at sacrifice is given. P, Proestrus; E, estrus; M, metestrus; D, diestrus.

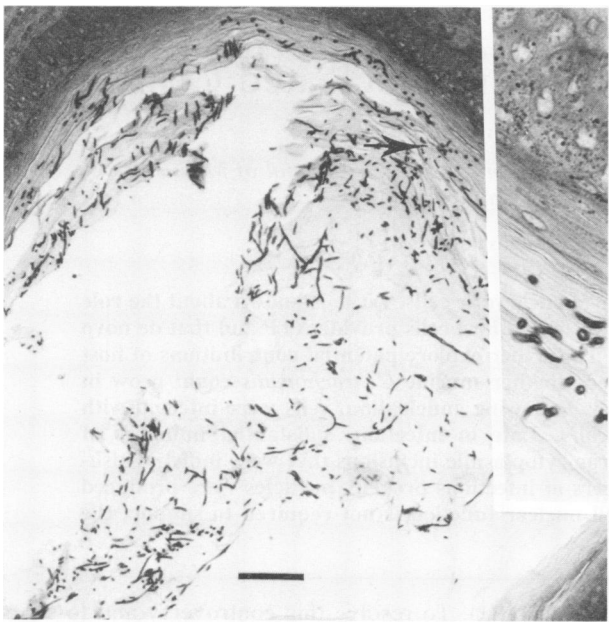

FIG. 1. Vagina of a *bg/bg nu/nu* mouse 14 days after intravaginal inoculation with *C. albicans*. *C. albicans* is shown infecting the outer keratinized layers of the vagina during late estrus. The arrow indicates the area shown magnitified in the inset. Bar, 100 μm.

candidiasis of the oral cavity, esophagus, and stomach (5) were not overtly susceptible to mucosal candidiasis of the genitourinary tract. It appears that the vaginas of congenitally immunodeficient mice are not easily naturally colonized or infected by *C. albicans*. Other environmental hormonal, nutritional, physiological, or immune factors must play an important role in susceptibility to *C. albicans* vaginitis. Congenitally immunodeficient gnotobiotic mice will be valuable animal models to study this important disease.

We thank Donna Brackett for typing the manuscript and Kristin Baggett for technical assistance.

This work was supported by Public Health Service grant DE-07339 from the National Institutes of Health, Bethesda, Md.

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