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Mucosal and Systemic Candidiasis in Congenitally Immunodeficient Mice

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Colony counts and light microscopy were used to assess the capacity of *Candida albicans* to colonize, infect the alimentary tract, and cause disseminated disease in athymic (*nu/nu*), euthymic (*nu/+*), beige (*bg/bg*), black (*bg/+*), beige athymic (*bg/bg nu/nu*), or beige euthymic (*bg/bg nu/+*) germfree mice. The alimentary tracts of all six genotypes of germfree mice were quickly colonized after exposure to yeast-phase *C. albicans*. Only *bg/bg nu/nu* mice showed obvious morbidity and mortality after mucosal colonization with *C. albicans*. Histopathology of *C. albicans*-colonized immunocompetent (*nu/+*, *bg/+*) and singly immunodeficient (*nu/nu*, *bg/bg*, *bg/bg nu/+*) mice showed minimal to moderate mucosal infections, whereas doubly immunodeficient (*bg/bg nu/nu*) mice showed extensive yeast and hyphal infection of the palate, tongue, esophagus, and stomach. A progressive systemic infection in *C. albicans*-colonized mice occurred only in *bg/bg nu/nu* mice 12 to 16 weeks after colonization and mucosal infection. Thus, it appears that a combination of defective cell-mediated immunity and phagocytic cell defects (polymorphonuclear leukocytes and/or macrophages) predisposed mice to severe mucosal and systemic candidiasis of endogenous origin. This is the first report of a mouse strain that is not only naturally susceptible to mucosal and systemic candidiasis of endogenous origin but also shows lethality at early (1 to 4 weeks) and late (12 to 16 weeks) times after alimentary tract colonization.

Candida albicans is a member of the normal flora of many mammals (32). The normal bacterial flora usually restricts the growth of *C. albicans* in the alimentary tract of immunocompetent animals (1, 32, 38). Congenital or iatrogenic immunosuppression often results in an overgrowth of endogenous populations of *C. albicans* that can cause cutaneous, mucosal (thrush, vaginitis, etc.), and systemic candidiasis (10, 12, 15, 30). Because of the increased use of immunosuppressive therapies for treating malignant diseases and immunological disorders, *C. albicans* infections have increased in incidence and importance (17, 24, 34, 41). Additional factors such as smoking, nutritional deficiencies, hormone imbalances, and trauma have also been implicated as predisposing factors for both systemic and mucosal candidiasis (1, 15, 33). A number of studies have attempted to clarify host defense mechanisms important for resistance to infections with *C. albicans* (2, 11, 14, 25, 29, 31, 35). Results from these studies have emphasized the complexity of the host-parasite interactions that occur with this fungus.

While a large amount of research has been directed at studying resistance to systemic candidiasis in rodents, relatively little research has been done on their resistance to mucosal candidiasis. Mucosal colonization of conventional adult animals with *C. albicans* can be achieved but requires a combination of immunosuppression, antibiotics, trauma, and inoculation with large numbers of *C. albicans* directly into the stomach (14, 16, 22). While conventional infant mice can be more readily colonized, they have mixed *C. albicans*-bacteria infections and mucosal thrushlike lesions do not develop (14, 16, 18, 22). It is worth noting that patients with mucosal candidiasis will very likely, like conventional animals, have mixed (*C. albicans*-bacteria) infections.

Germfree (GF) animals are uniquely suited for studies of mucosal candidiasis. Mucosal surfaces of adult GF animals can be naturally and chronically colonized by *C. albicans* without the need for trauma or immunosuppressive and antimicrobial therapy (3, 4). GF mice are valuable tools for studying host-*C. albicans* interactions in pure culture at mucosal surfaces. Furthermore, the mucosal colonization established in GF mice persists for the lifetime of the animal (3, 4).

There are a number of animals which are genetically immunodeficient and manifest defects in innate immunity or cell-mediated immunity or both (21, 37, 40, 42, 43). Balish et al. (3, 4) have established that GF BALB/c *nu/nu* mice colonized with *C. albicans* have chronic mucosal infections (hyphal penetration) of keratinized portions of the stomach and tongue. The latter study with athymic mice supports observations made in humans, which associated mucosal candidiasis with defective cell-mediated immunity responses (23, 38). We documented more completely the mucosal *C. albicans* infection established in *nu/nu* and *nu/+* GF mice and assessed the susceptibility of GF *bg/bg*, *bg/+*, *bg/bg nu/nu*, and *bg/bg nu/+* mice to mucosal candidiasis. The aim of this work was to characterize the susceptibility of congenitally immunodeficient GF mice to mucosal and systemic candidiasis of endogenous origin.

MATERIALS AND METHODS

Microorganism. *C. albicans* B311 (type A) was maintained by monthly transfers on Sabouraud dextrose agar (SDA) slants. Yeast cells were cultured for 24 h at 37°C on SDA and then stored at 4°C. For experiments, a single stock was prepared with organisms grown on SDA for 24 h at 37°C and washed from the slants (10^8/ml).

Mice. Congenitally immunodeficient mice were produced by mating homozygous (*nu/nu*, *bg/bg*, or *bg/bg nu/nu*) males with heterozygous (*nu/+*, *bg/+*, and *bg/bg nu/+*, respectively) females.

GF mice were originally derived from NIH BALB/c nude mice, NIH C57BL/6 beige mice, and N:NIH(S) III beige athymic mice by cesarean derivation and have since been

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TABLE 1. Colonization of congenitally immunodeficient mice with *C. albicans*

Wk after MA[a]	CFU of *C. albicans* per g[b]					
	nu/nu	*nu/+*	*bg/bg*	*bg/+*	*bg/bg nu/nu*	*bg/bg nu/+*
2	6.3 ± 1.2	6.6 ± 0.2	7.6 ± 0.1	8.1 ± 0.4	7.4 ± 0.2	7.7 ± 0.1
4	7.3 ± 0.4	7.2 ± 0.1	7.9 ± 0.3	7.9 ± 0.1	7.1 ± 0.2	8.3 ± 0.1
9	8.0 ± 0.3[c]	6.7 ± 0.3	8.7 ± 0.1[c]	7.9 ± 0.1	7.8 ± 0.3	8.3 ± 0.1
11–13	8.3 ± 1.1	6.9 ± 0.2	7.0 ± 0.3	6.3 ± 0.2	8.1 ± 0.3	8.1 ± 0.1
15–16	7.3 ± 0.5	7.5 ± 0.7	8.7 ± 0.1	8.0 ± 0.3	8.4 ± 0.2	7.3 ± 0.1

[a] MA, Weeks of alimentary tract colonization with a pure culture of *C. albicans*.
[b] Mean $\log_{10}$ *C. albicans* CFU per gram (dry weight) ± standard error of the mean. Counts represent the mean of at least three mice per sacrifice interval.
[c] Immunodeficient mice had statistically larger numbers of *C. albicans* in their stomachs than their immunocompetent littermates (significant to 95% or $P < 0.025$).

bred and housed in flexible film isolators at the University of Wisconsin Gntobiotic Research Laboratory (Madison). The GF or *C. albicans*-colonized status of each experiment was assessed by methods previously described (5).

Confirmation of immune defects in congenitally immunodeficient mice. The functions of granulated leukocytes, T cells, and B cells from our immunodeficient mice were characterized. Beige mouse defects in granulated leukocytes were characterized in two ways. (i) Natural killer cell activity of splenic lymphocytes was measured in a standard 4-h assay with chromated YAC-1 tumor targets, and (ii) microscopic observations of embedded tissue sections were made to assess the size of granules within granulated cells (6, 7, 20, 36). Defects in functional T cells from nude mice were assessed by assaying the mitogenic response of their splenocytes to the T-cell mitogen concanavalin A in a lymphocyte proliferation assay (4, 20, 28, 39). B-cell function was screened in our mice by using a spleen cell proliferation assay and the B-cell mitogen lipopolysaccharide (4, 28, 43). The beige defect (abnormally large granules) was observed with lymphocytes from *bg/bg*, *bg/bg nu/+*, and *bg/bg nu/nu* mice but not with lymphocytes from *bg/+*, *nu/+*, or *nu/nu* mice. The nude mouse defect in functional T cells was only observed in *nu/nu* and *bg/bg nu/nu* mice. All mouse strains were found to have B cells which responded to lipopolysaccharide in a lymphocyte proliferation assay.

Monoassociation. Adult GF mice were orally inoculated by mixing *C. albicans* in their drinking water at a concentration of approximately 10^5 viable *C. albicans* per ml. GF mice were subsequently transferred into flexible film isolators that contained mice colonized with a pure culture of *C. albicans*. Adult GF mice, perhaps because they are coprophagic, are quickly (24 h) colonized by natural exposure to other *C. albicans*-colonized mice and their contaminated cage bedding (3, 36).

Enumeration of viable *C. albicans*. Mice were either sacrificed in groups of three by ether inhalation or necropsied shortly (up to 5 h) after death (NSAD) resulting from *C. albicans* colonization. The organs were aseptically removed and placed in 5 ml of saline. Each organ was homogenized separately, and dilutions were made of each homogenate. The number of viable *C. albicans* was determined by plating on SDA. Colonies were counted after 24 h of incubation at 37°C. The viable units of *C. albicans* were expressed as the number of CFU per gram (dry weight) of organ. Data shown are from repeat experiments. Representative colonies were routinely confirmed as *C. albicans* by microscopic observations, biochemical reaction, colony morphology on SDA, and germ-tube formation in serum.

Histology. Tissues were excised and immediately placed into Hollande-Bouin fixative. The 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. Tissues were all cut and embedded in the same way to ensure sampling of similar areas for each time point and mouse strain. Multiple stained sections from each embedded tissue were viewed under light microscopes. The organs from at least two mice were embedded, cut, and viewed for each data point reported. Tissue sections in Table 5 and in the figure legends were scored as 0 to 4 for the degree to which *C. albicans* hyphae infected the mucosal surfaces. Criteria for mucosal infectivity scores were as follows: 0, no hyphal penetration of mucosal surfaces; 1, sporadic yeasts and hyphae; 2, numerous yeasts and hyphae; 3, abundant yeasts and hyphae but not confluent; and 4, confluent invasion of mucosal surfaces with yeasts and hyphae. *C. albicans* yeasts were often seen in the lumen of the intestinal tract, but only *C. albicans* hyphal penetration of mucosal surfaces or yeast invasion of the viable epithelium was included in the mean mucosal infectivity scores.

TABLE 2. Disseminated candidiasis in *C. albicans*-colonized gnotobiotic nude (*nu/nu*) and heterozygous (*nu/+*) BALB/c mice

Wk after MA[a]	No. of viable *C. albicans*[b] in:							
	Kidney		Liver		Spleen		MLN	
	nu/nu	*nu/+*	*nu/nu*	*nu/+*	*nu/nu*	*nu/+*	*nu/nu*	*nu/+*
2	0	1.3 ± 0.7	0	0.8 ± 0.8	0	0	2.2 ± 1.4	3.4 ± 1.7
4	0	0	0	0	0	0	1.3 ± 1.3	0
9	0.6 ± 0.6	3.4 ± 1.4	0.4 ± 0.4	0.9 ± 0.9	0	1.3 ± 1.3	4.3 ± 0.4	4.1 ± 0.4
11	0	0.5 ± 0.5	1.21 ± 1.21	0	0	0	0	1.9 ± 1.2
13	0	0.7 ± 0.7	0	0.6 ± 0.6	0	0	1.5 ± 0.8	1.5 ± 1.5
15	1.0 ± 1.0	0	0.8 ± 0.8	0	1.0 ± 1.0	0	1.9 ± 1.9	3.0 ± 1.6

[a] MA, Weeks of alimentary tract colonization with a pure culture of *C. albicans*.
[b] Mean $\log_{10}$ *C. albicans* CFU per gram (dry weight) ± standard error of the mean. Counts represent the mean of three mice per sacrifice interval.

TABLE 3. Disseminated candidiasis in *C. albicans*-colonized gnotobiotic beige (*bg/bg*) and black (*bg/+*) C57BL mice

Wk after MA[a]	No. of *C. albicans*[b] in:							
	Kidney		Liver		Spleen		MLN	
	bg/bg	*bg/+*	*bg/bg*	*bg/+*	*bg/bg*	*bg/+*	*bg/bg*	*bg/+*
2	1.2 ± 0.9	0.8 ± 0.6	1.8 ± 0.8	1.5 ± 0.8	0	0	2.6 ± 1.1	6.1 ± 0.8
4	0.6 ± 0.5	1.3 ± 1.1	0.5 ± 0.5	0.5 ± 0.5	0	0	3.7 ± 0.1	5.1 ± 0.5
9	1.8 ± 1.8	0	0	0	0	0	0.8 ± 0.8	0
12	0	0.6 ± 0.6	0	0	0	0.8 ± 0.8	1.0 ± 1.0	2.5 ± 0.5

[a] MA, Weeks of alimentary tract colonization with a pure culture of *C. albicans*.
[b] Mean $\log_{10}$ *C. albicans* CFU per gram (dry weight) ± standard error of the mean. Counts represent the mean of three animals per sacrifice interval.

Mean mucosal infectivity scores were rounded to the closest whole number.

Statistical analysis. The Student *t* test ($P < 0.025$ to $P < 0.005$, 95 to 98% confidence intervals) or the analysis of variance test (significant at 95%) were used to test for significant differences in the mean number of *C. albicans* isolated from mouse tissues.

RESULTS

Colonization of GI tract. Populations of viable *C. albicans* present in the stomach, small intestine (SI), cecum, and colon of *nu/nu*, *nu/+*, *bg/bg*, *bg/+*, *bg/bg nu/nu*, and *bg/bg nu/+* mice colonized with *C. albicans* for 2 to 16 weeks were assessed by plate counts of homogenized gut sections. Culturing the gastrointestinal (GI) tract at several time intervals over a 16-week period showed that 6 to 8 $\log_{10}$ *C. albicans* CFU/g (dry weight) were recoverable from the stomach, SI, cecum, and colon of all mice over the 16-week time period (Table 1; data from SI, cecum, and colon not shown). Occasionally, immunodeficient animals appeared to have significantly larger numbers of *C. albicans* in their stomachs when compared with their immunocompetent littermates (Table 1). However, we observed no consistently increased numbers of viable *C. albicans* in the GI tract of any strain of immunodeficient mouse used. The 1 to 2 $\log_{10}$ differences we occasionally observed in counts could be due to differences in the amount of material (mucus, sloughed-off colonized or infected tissues, etc.) in the GI tract or perhaps fragmentation of hyphal forms to a greater extent in some homogenizations than others. Regardless of genotype or mouse strain, the GI tracts of all the gnotobiotic mice were persistently colonized with very large numbers of a pure culture of *C. albicans* for the duration of our experiments.

Systemic candidiasis of endogenous origin. The liver, kidneys, spleen, and mesenteric lymph nodes (MLN) of *nu/nu*, *nu/+*, *bg/bg*, *bg/+*, *bg/bg nu/+*, and *bg/bg nu/nu* *C. albicans*-colonized mice were also cultured for viable *C. albicans* over a 16-week experimental period. Viable *C. albicans* was consistently isolated from the MLN of all six mouse genotypes (Tables 2, 3, and 4). *C. albicans* appeared to readily translocate to the MLN; however, a progressive systemic infection did not occur in *bg/bg*, *bg/+*, *nu/nu*, *nu/+*, and *bg/bg nu/+* mice as evidenced by sporadic but low numbers of viable *C. albicans* cultured from the internal organs in these mice with time after colonization (Tables 2, 3, and 4). Only in *bg/bg nu/nu* mice were increasing numbers of *C. albicans* found in the MLN and in the internal organs with time after colonization, indicating that a progressive disseminated infection was occurring in these *bg/bg nu/nu* mice. At 12 and 16 weeks of colonization, the *bg/bg nu/nu* mice had significantly higher numbers of *C. albicans* in their internal organs than their *bg/bg nu/+* littermates (Table 4).

Lethality of *C. albicans* for *bg/bg nu/nu* mice. *nu/nu*, *nu/+*, *bg/bg*, *bg/+*, and *bg/bg nu/+* mice did not die after colonization with a pure culture of *C. albicans* over a 32-week period. Conversely, colonization with *C. albicans* was associated with mortality in *bg/bg nu/nu* mice. Of 73 mice, 22 (30%) died within 4 weeks of colonization with *C. albicans*. The latter mortality value is very likely a low estimate because a large number of the 73 *C. albicans*-colonized mice were sacrificed for culturing and histology. Mice that died during the first 4 weeks after *C. albicans* colonization appeared to have been wasting, and necropsy revealed hemorrhaging of the stomach and small intestine. Necropsied *bg/bg nu/nu* mice (which died after 1 to 4 weeks of colonization with *C. albicans*) had visible lesions in their oral cavities, while their internal organs showed no macroscopic signs of infection. The low numbers of viable *C. albicans* in the internal organs of *bg/bg nu/nu* mice (cultured shortly after their death) did not appear to be sufficient to explain the

TABLE 4. Disseminated candidiasis in *C. albicans*-colonized gnotobiotic beige athymic (*bg/bg nu/nu*) and beige euthymic (*bg/bg nu/+*) NIH(S) III mice

Wk after MA[a]	No. of mice		No. of *C. albicans*[b] in:							
	bg/bg nu/nu	*bg/bg nu/+*	Kidney		Liver		Spleen		MLN	
			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/+*
2	6	5	0	0.3 ± 0.3	0.4 ± 0.4	0.9 ± 0.4	0	0.5 ± 0.5	2.4 ± 0.8	1.9 ± 0.8
4	4	3	1.2 ± 0.6	1.0 ± 0.5	1.4 ± 0.6	0.9 ± 0.9	0.7 ± 0.7	0	1.4 ± 0.9	1.8 ± 1.0
9	3	5	0	1.3 ± 0.8	2.5 ± 0.9	1.2 ± 0.7	1.5 ± 1.2	0.9 ± 0.9	3.9 ± 1.6	1.5 ± 0.9
12	6	6	2.0 ± 1.2[c]	0	1.8 ± 0.5[c]	0	1.5 ± 0.7[c]	0	4.7 ± 0.6[c]	1.9 ± 1.0
16	6	3	3.2 ± 1.0[c]	1.5 ± 0.9	3.0 ± 0.5[c]	0	4.2 ± 0.5[c]	0	3.8 ± 0.2[c]	1.8 ± 0.9

[a] MA, Weeks of alimentary tract colonization with a pure culture of *C. albicans*.
[b] Mean $\log_{10}$ *C. albicans* CFU per gram (dry weight) ± standard error of the mean.
[c] *bg/bg nu/nu* mice had significantly higher numbers of *C. albicans* in their internal organs than their *bg/bg nu/+* littermates ($P < 0.005$ to 0.001).

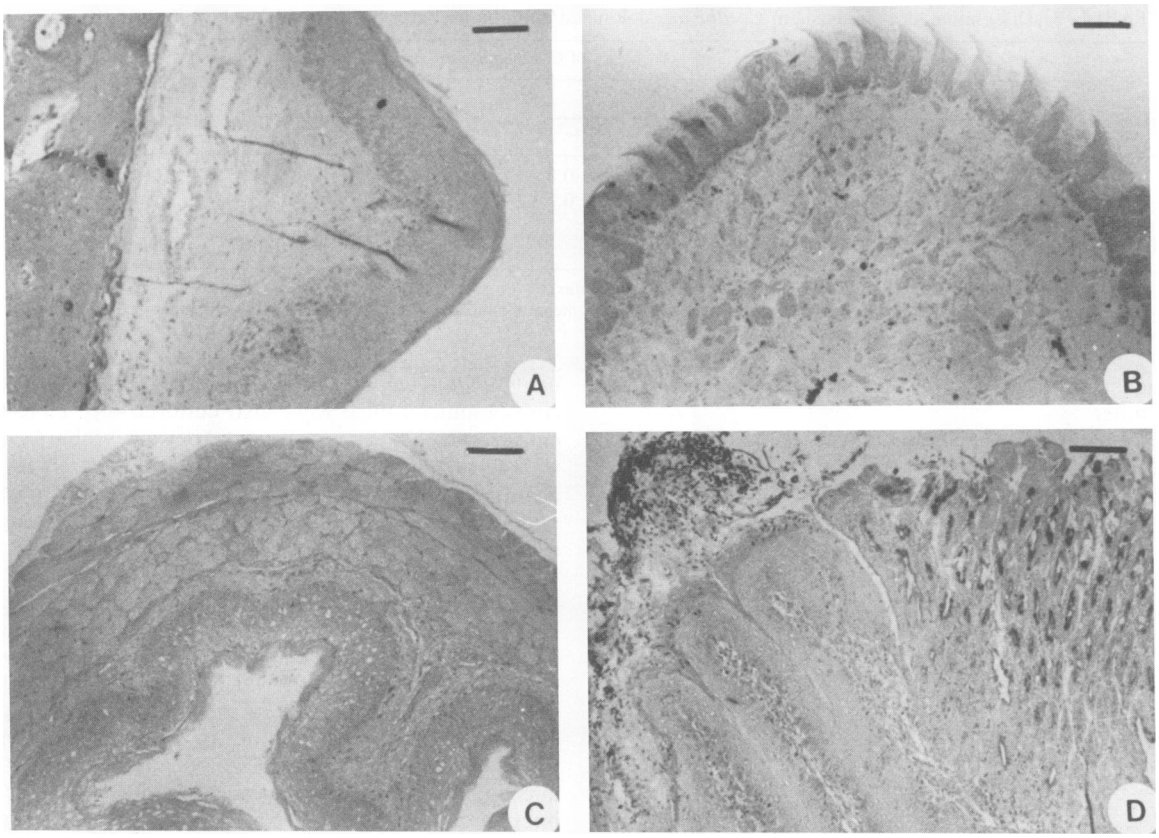

FIG. 1. *bg/bg nu/+* mice were killed and necropsied after *C. albicans* colonization. The palate (A; 2 weeks of colonization), tongue (B; 2 weeks of colonization), esophagus (C; 2 weeks of colonization), and stomach (D; 12 weeks of colonization) were sectioned and stained with periodic acid-Schiff and azure A-eosin B or hematoxylin and eosin. (A, B, and C) Tissue sections of the palate, tongue, and esophagus showed no infection (scored as 0). (D) *C. albicans* yeasts and hyphae are shown infecting the keratinized secretory junction of the stomach (scored as 2). Bar, 100 μm.

morality that occurred shortly after colonization (1 to 4 weeks [Table 4]; NSAD mice culture data not shown). Deaths of immunodeficient *bg/bg nu/nu* mice that survived beyond 4 weeks of colonization were also observed after longer periods of colonization (12 to 14 weeks). *bg/bg nu/nu* mice that died 12 to 14 weeks after *C. albicans* colonization had 4 to 7 $\log_{10}$ CFU/g (dry weight) of *C. albicans* in their internal organs. Disseminated candidiasis appeared to be more pronounced in *bg/bg nu/nu* mice colonized for 12 or more weeks, and thus disseminated systemic candidiasis could explain the lethality which occurred in mice colonized with *C. albicans* for long periods (i.e., beyond 4 weeks [Table 4]).

Histopathology of *C. albicans*-colonized alimentary tract. Culture data from homogenized tissues indicated that *C. albicans* persisted in the alimentary tract. Histology of alimentary tract tissues indicated the extent of *C. albicans* infection (hyphal penetration) of mucosal surfaces. Sections of the stomach, tongue, and esophagus were saved from all mouse strains either shortly after an animal died or at the time that animals were killed (1 to 33 weeks after *C. albicans* colonization). The hard palate, Peyer's patches (PP), and SI of selected mice were also removed, fixed, sectioned, stained, and screened for histopathology.

Table 5 summarizes our observations on temporal aspects of the severity of *C. albicans* infection observed in the esophagi and tongues of *bg/bg nu/nu* and *bg/bg nu/+* mice and moribund or dead *bg/bg nu/nu* mice (NSAD).

Oral cavity. Histology of tongues from immunocompetent and singly immunodeficient mice colonized with *C. albicans* primarily showed low numbers of *C. albicans* colonizing the outer keratinized layers of the tongue with little mucosal infection (represented by a *bg/bg nu/+* tongue in Fig. 1B). The hard palates and esophagi from immunocompetent and singly immunodeficient mice showed no signs of infection (represented by a *bg/bg nu/+* palate and esophagus in Fig.

FIG. 2. *bg/bg nu/nu* mice which died (NSAD) after *C. albicans* colonization were necropsied. The palate (A; 2 weeks of colonization), tongue (B; 2 weeks of colonization), esophagus (C; 2 weeks of colonization), and stomach (D; 14 weeks of colonization) were sectioned and stained with periodic acid-Schiff and azure A-eosin B or hematoxylin and eosin. Arrows indicate areas shown magnified in the insets. (A) Pseudomembranous layer of *C. albicans* is seen coating the surface of the hard palate (scored as 4). (B) *C. albicans* yeasts and hyphae are shown infecting the dorsal surface of the tongue with some inflammation (scored as 4). The dark-staining area surrounding the white asterisks is a thick layer of *C. albicans* yeasts and hyphae. (C) Esophagus shows *C. albicans* yeasts and hyphae which are occluding the esophagus with minimal inflammation (scored as 4). (D) Extensive penetration of *C. albicans* yeasts into the secretory portion of the stomach (scored as 4). Bar, 100 μm.

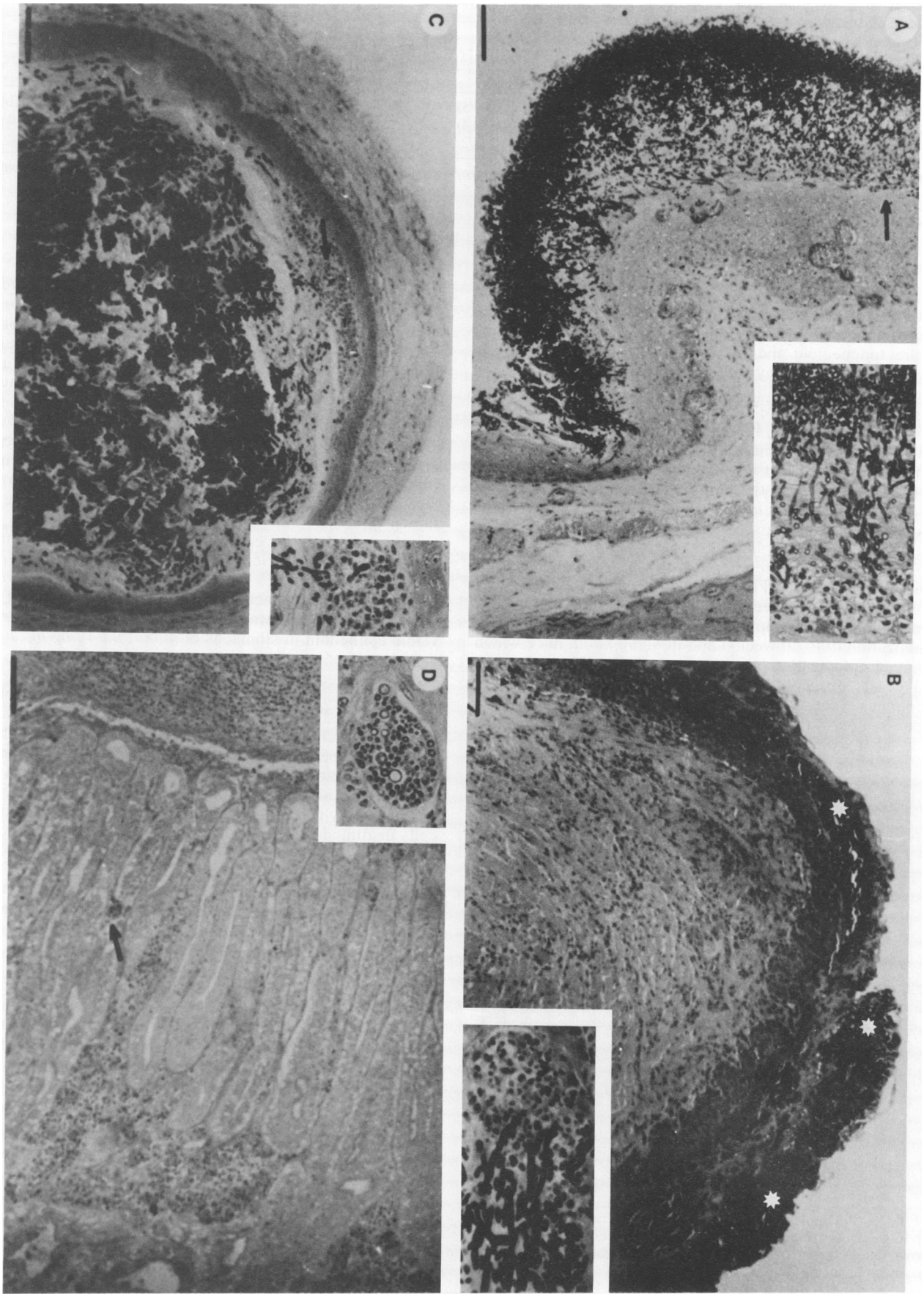
C
A
D
B

TABLE 5. Candidiasis of the tongue and esophagus in congenitally immunodeficient mice[a]

Wk after MA[b]	Tongue			Esophagus		
	NSAD[c] *bg/bg nu/nu*	*bg/bg nu/nu*	*bg/bg nu/+*	NSAD *bg/bg nu/nu*	*bg/bg nu/nu*	*bg/bg nu/+*
1–4	4.0 (7/7)	2.0 (6/7)	0 (0/5)	4.0 (7/7)	1.0 (7/10)	0 (1/5)
12–16	2.0 (2/2)	1.0 (4/4)	0 (0/4)	0 (0/2)	1.0 (4/4)	0 (0/4)
20–32	ND[d]	1.0 (2/2)	0 (0/2)	ND	1.0 (2/2)	0 (0/2)

[a] Tissue sections were visually rated (0 to 4) for the mucosal infectivity (see Materials and Methods) (number of mice infected/number of mice examined). Mucosal infectivity scores were rounded to the nearest whole number.
[b] MA, Weeks of alimentary tract colonization with a pure culture of *C. albicans*.
[c] NSAD, Necropsied shortly after death.
[d] ND, Not done.

1A and C, respectively). In stark contrast, *bg/bg nu/nu* mice that died at 2 weeks after colonization (NSAD) showed macroscopic plaquelike lesions covering the dorsal surface of the tongue and the hard palate. Microscopically, *C. albicans* yeasts and hyphae were seen in large numbers attached to and penetrating the keratinized portions of the tongue and hard palate (Table 5; Fig. 2A and B). Normal filiform papillae usually found on the tongue (seen in Fig. 1B) were replaced by a thick layer of *C. albicans* yeasts and hyphae (Fig. 2B). Little inflammation of the palate and tongue was evident in these *bg/bg nu/nu* mice (Fig. 2A and B). The esophagi from *bg/bg nu/nu* NSAD mice (2 weeks of colonization) showed pseudomembranous layers of *C. albicans* which in some places occluded the esophogus (Fig. 2C). This is the first report of esophageal candidiasis in a murine model. Hyperkeratosis of the palate, tongue, and esophagus was observed in *bg/bg nu/nu* mice that died 2 weeks after colonization (NSAD). At longer periods after colonization (9 to 20 weeks) and in *bg/bg nu/nu* mice which survived colonization, the numbers of *C. albicans* hyphae and yeasts observed adhering to the palate, tongue, and esophogus were greatly decreased, as was the amount of hyperkeratosis (Table 5; histology not shown).

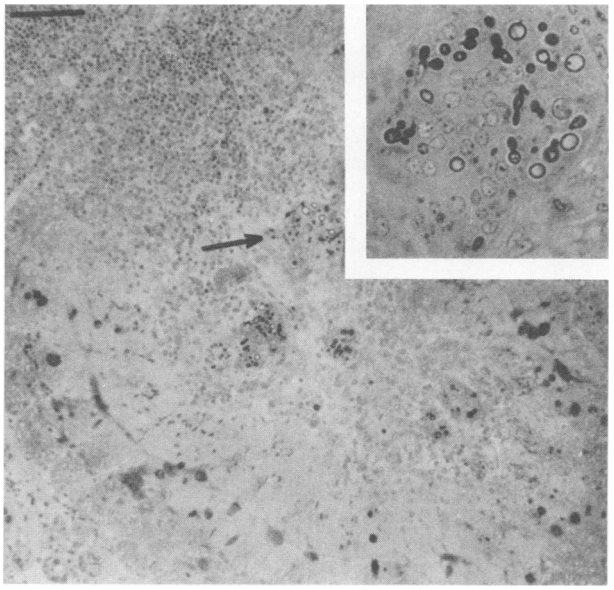

FIG. 3. PP from a *bg/bg nu/nu* mouse colonized with *C. albicans* for 16 weeks (scored as 1.0). Giant cells containing *C. albicans* are shown within the PP. The arrow indicates the giant cell magnified in the inset. Bar, 100 μm.

Stomach. *C. albicans* infections of the stomach at 2 weeks of colonization (in all strains of mice) were restricted to the junction of keratinized and secretory tissues. After longer periods of colonization (e.g. 4, 12, and 24 weeks), immunocompetent (*nu/+*, *bg/+*) mice still only showed minimal infections of their stomachs. Conversely, in singly immunodeficient mice a low-grade infection persisted (over 32 weeks of colonization). Figure 1D shows the stomach of a *bg/bg nu/+* mouse colonized for 12 weeks, which was representative of stomachs from singly immunodeficient mice which become chronically infected with low numbers of organisms. In Fig. 1D, *C. albicans* yeasts and hyphae are shown infecting only the proximal keratinized tissue surrounding the keratinized-secretory junction. Occasionally, in singly immunodeficient mice colonized with *C. albicans* (12 or more weeks), *C. albicans* was also observed loosely attached to the surface of the secretory portion of the stomach. *C. albicans* was apparently unable to penetrate the viable epithelium in stomachs of *nu/nu*, *nu/+*, *bg/bg*, *bg/+*, or *bg/bg nu/+* mice.

Stomach sections from doubly immunodeficient (*bg/bg nu/nu*) mice which died or were killed at 2 weeks of colonization showed *C. albicans* infecting only the keratinized-secretory junction of the stomach and only in low numbers. After longer periods of colonization (12 to 16 weeks), necrosis of the keratinized portions of *bg/bg nu/nu* stomachs was evident and the secretory portions of these stomachs were penetrated by *C. albicans*. A great number of inflammatory cells disrupted the stomach framework. *C. albicans* yeasts (not hyphae) were observed within the viable tissues of the gastric crypts in these stomach sections (Fig. 2D).

SI and PP. Because the small intestine (SI) from *bg/bg nu/nu* NSAD mice (2 weeks of colonization) contained pooled blood and accumulated gas, some SI were also sectioned and examined for histopathology. A few *C. albicans* yeast forms were observed in the lumen of SI from *bg/bg nu/nu* NSAD mice, and the lamina propria showed decreased cellularity and a great deal of necrotic tissue when compared with SI from *bg/bg nu/+* mice colonized with *C. albicans* for 2 weeks (histology not shown). While the SI was sectioned, Peyer's patches (PP) were also observed and sectioned. *C. albicans* was not evident in the PP at 2 and 9 weeks (two mice were sampled at both 2 and 9 weeks) of colonization; however, at 16 weeks of colonization, *C. albicans* was observed within giant cells in the PP of *bg/bg nu/nu* mice (Fig. 3).

DISCUSSION

This study demonstrates that gnotobiotic *bg/bg nu/nu* mice are naturally susceptible to mucosal and systemic candidiasis and provides an excellent animal model to study

various aspects of susceptibility and resistance to this disease. To our knowledge, no other animal model has been described that is naturally susceptible to chronic mucosal and systemic candidiasis of endogenous origin. Previous experimental infections of the mucosa after oral challenge with *C. albicans* have been limited to low numbers of yeasts and hyphae colonizing the surface of the palate, tongue, and stomach (3, 27). The *bg/bg nu/nu* mouse is the first animal model of candidiasis that manifests natural susceptibility to hard palate and esophageal candidiasis. The pseudomembranous layer of *C. albicans* lining the oral cavity and esophagus in *bg/bg nu/nu* mice is very reminiscent of clinical thrush observed in patients with acquired immunodeficiency syndrome who have mucosal candidiasis (24, 34).

The mucosal surfaces of singly immunodeficient and immunocompetent mice did manifest infections with *C. albicans*, but the infections were not as severe as those observed in *bg/bg nu/nu* mice. Little to no infection of the hard palate, tongue, or esophagus was evident in singly immunodeficient and immunocompetent mice. *C. albicans* infections in the stomachs of immunocompetent (*bg/+*, *nu/+*) mice are apparently controlled and in some cases may even be cleared. Stomachs from singly immunodeficient mice developed chronic low-grade infections with *C. albicans*.

Approximately 30% of the *bg/bg nu/nu* mice died during the first 4 weeks after colonization with *C. albicans*. These mice had a very large number of organisms infecting their oral cavity and esophagus, and little inflammation was evident in the infected tissues. Tongue and esophageal sections from mice that survived beyond 4 weeks of colonization and those that were killed at 1 to 4 weeks after colonization had fewer *C. albicans* hyphae and yeasts associated with their tongues and esophagi and more pronounced mucosal inflammatory responses (primarily polymorphonuclear leukocytes). Although *bg/bg nu/nu* mice were very susceptible to mucosal candidiasis, even some of these mice were apparently able to mount an inflammatory response. As the time of colonization increased, the lesions on the tongues and esophagi of *bg/bg nu/nu* mice appeared to improve, whereas candidiasis of the stomach appeared to be chronic since no resolution of this infection was evident over the 32-week study.

The cause of early death in *bg/bg nu/nu* mice was probably not systemic spread of *C. albicans*. Necropsy of NSAD *bg/bg nu/nu* mice showed hemorrhaging and microscopic areas of necrosis in the stomachs and SI of these mice. *bg/bg nu/nu* mice which died 1 to 4 weeks after colonization may not have been able to elicit an inflammatory response early enough to stop the overgrowth of *C. albicans* in the oral cavity. The increased numbers of organisms and the mucosal infections may have interfered with food consumption in moribund mice and/or may have resulted in the production of toxic compounds that caused the necrosis observed in the stomach and SI. Mice which were apparently healthy at 4 weeks of colonization appeared to have mounted an inflammatory response, and this may have helped to control the oral and esophageal infections. However, these *bg/bg nu/nu* mice were never able to resolve the infection in their stomachs, which was chronic and persisted for the duration of the study. The gastric infection not only spanned both the secretory and keratinized portions of the *bg/bg nu/nu* stomach, but *C. albicans* was also visible within the viable tissue in the PP (16 weeks of colonization) located on the SI. Apparently, the large numbers of *C. albicans* in the alimentary tract could not be controlled by the immunodeficient (*bg/bg nu/nu*) host, reached the PP, and eventually broke through into systemic circulation. It seems likely that with time all these *bg/bg nu/nu* mice eventually would succumb to complications resulting from the spread of *C. albicans* into the systemic circulation.

It appears that a combination of defects in both thymus-matured T cells (cell-mediated immunity) and innate immunity (phagocytes) predisposes the *bg/bg nu/nu* mouse to mucosal and systemic candidiasis of endogenous origin. Mucosal surfaces of mice with a single congenital immunodeficiency (either beige or nude) became only moderately infected, and progressive systemic candidiasis was not evident. It therefore seems likely that T-cell–phagocytic cell interactions are important for resistance to mucosal and systemic candidiasis. Resistance of *bg/bg* and *bg/bg nu/+* mice could be explained by the fact that their T cells were producing factors which help overcome the genetic defects in their phagocytic cells (9). Such T-cell–phagocytic cell interactions could be cytokine mediated, perhaps by gamma interferon, which has been shown to augment the *C. albicans* growth-inhibiting activities of phagocytes (13). The lack of this T-cell–phagocytic cell interaction could explain the susceptibility of the *bg/bg nu/nu* mice to mucosal and systemic candidiasis of endogenous origin.

Genetic factors have been shown by a number of groups to play a role in resistance to experimental systemic candidiasis (8, 19, 26). C57BL/6 mice apparently carry a resistance phenotype which makes them less susceptible than BALB/c mice to systemic candidiasis (26). From our study, it was clear that the immune competence of the mouse was more important than the strain of the mouse in determining susceptibility to mucosal candidiasis. Contrary to what is seen with systemic candidiasis, the *C. albicans* mucosal infection in C57BL/6 (*bg/+*) mice was no more or less severe than in BALB/c (*nu/+*) mice. Our work with mice confirms observations by other investigators who showed that different immunodeficiencies are associated with differences in susceptibility to systemic and mucosal candidiasis in humans (1, 10, 12, 25, 38). It appears that in mice, as in humans, the immune status of the host is an important factor in susceptibility to mucosal and systemic candidiasis of endogenous origin (1, 3, 17, 23, 25, 30, 34).

Defective T-cell-mediated immunity is common in patients with mucocutaneous candidiasis (23, 34, 41). In addition, patients who become infected with *C. albicans* often undergo treatments that can affect phagocytic cells function (23, 41). The *bg/bg nu/nu* mouse mimics both of these defects, and mucosal candidiasis observed in these mice closely mimics clinical thrush. To our knowledge, the *bg/bg nu/nu* mouse is the first animal model to be naturally susceptible to mucosal and systemic candidiasis of endogenous origin. Putative therapeutic agents for mucocutaneous as well as systemic candidiasis can readily be tested in this animal model. The *bg/bg nu/nu* mouse will be an extremely valuable tool for future studies of *C. albicans*-host interactions.

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