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Correlates of Cell-Mediated Immunity in *Candida albicans*-Colonized Gnotobiotic Mice

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Germfree athymic (*nu/nu*) and euthymic (*nu/+*) mice were colonized with a pure culture of *Candida albicans*. Correlates of cell-mediated immunity (lymphocyte proliferation and footpad responses to *C. albicans* antigens) and in vivo clearance of mucosal infections were assessed at different time intervals after alimentary tract colonization. *C. albicans* hyphae infected the dorsal surface of the tongue and the cardial section of the stomach in both *nu/nu* and *nu/+* mice within 1 week after colonization with a pure culture of *C. albicans*. With time after colonization and infection with *C. albicans*, *nu/+* mice manifested positive lymphocyte proliferation and positive footpad responses to *Candida* antigens that appeared to correlate with the capacity to clear *Candida* hyphae from the dorsal surface of the tongue and in the stomach. Conversely, *nu/nu* mice could not clear mucosal candidosis (in the stomach and on the tongue) and did not manifest either lymphocyte proliferation or footpad swelling in response to *C. albicans* antigens. These studies indicated that T-cell-mediated immunity may play a role in the acquired resistance of mice to mucosal candidosis. Since neither *nu/nu* nor *nu/+* mice developed a progressive systemic disease, T cells apparently do not play a prominent role in murine resistance to systemic candidosis of endogenous origin.

Candida albicans is commonly isolated from mucosal surfaces (3, 10) and can stimulate antibody- and cell-mediated immune responses in humans (2, 8, 19, 23–25, 27) and animals (1, 4, 6, 7, 11, 14, 22). It is not known whether alimentary tract colonization alone or a combination of mucosal and systemic infections is required for *C. albicans* to stimulate the cellular and humoral immune mechanisms of a host (22, 23). Very few studies have assessed the immune responses of a host following alimentary tract colonization with *C. albicans* because it is very difficult to chronically colonize adult conventional animals with large viable populations of *C. albicans* (1, 12). Antibiotics, trauma, and immunosuppressive or cytotoxic drugs are used to interfere with inhibitory microbial flora, damage tissues, or immunosuppress the host (1, 4, 13, 26) in order to predispose it to mucosal candidosis. The use of antibiotics and immunosuppressive agents complicates data on host immunity to *C. albicans* because of adverse effects on alimentary tract ecology, interference with the cellular and humoral immunocompetence of the host, and enhanced susceptibility to bacterial infections (4, 6, 9, 26, 27).

Several investigators have assessed the cellular and humoral immune mechanisms of *C. albicans*-colonized mice. In 1974, Molinari et al. (18) inoculated the alimentary tracts of germfree (GF) mice with killed *C. albicans* and observed that mice formed secretory antibodies (immunoglobulin A) against *C. albicans*. Balish et al. (1) demonstrated that gnotobiotic euthymic (*nu/+*) BALB/c mice colonized with a pure culture of *C. albicans* cleared an intravenous challenge with *C. albicans* from their kidneys at a faster rate than did monoassociated (MA) athymic (*nu/nu*) gnotobiotic mice. Recent studies demonstrated that mice intubated with large inocula of *C. albicans* as neonates were primed for cellular and humoral immune responses to *Candida* antigens (5, 17). In addition, the inoculation of *C. albicans* into the stomachs of adult conventional mice resulted in minimal immune responses to *Candida* antigens and some protective immunity against a systemic challenge with *C. albicans* (6). Since intubation with large inocula results in systemic candidosis in rodents (neonates and adults) and humans (6, 7, 16, 17, 20, 21), mucosal infections or systemic spread or both, in addition to alimentary tract colonization, may be needed for *C. albicans* to evoke cellular and humoral immune responses in mice.

In this study, we assessed several correlates of cell-mediated immunity in gnotobiotic *nu/nu* and *nu/+* mice that were either colonized (nontraumatic inoculation) as neonates or as adults with a pure culture of *C. albicans*. The pathogenesis of this yeast for *nu/nu* and *nu/+* mice was assessed by counts of viable *C. albicans* present in tissues and organs with time after challenge and by histology of tissue sections stained with periodic acid-Schiff or hematoxylin and eosin.

MATERIALS AND METHODS

Microorganism. *C. albicans* B311 (type A) was maintained in our laboratory by monthly transfers on Sabouraud dextrose agar slants. For experiments, organisms were grown on Sabouraud dextrose agar for 24 h at 37°C, washed with phosphate-buffered saline (PBS) (0.1 M; pH 7.4) from the slants, centrifuged (500 × *g* for 15 min), counted in a hemacytometer, and diluted in PBS to achieve the desired number of viable *C. albicans* per milliliter. The intravenously administered 50% lethal dose of this *C. albicans* strain is 2×10^4 for BALB/c *nu/+* mice.

Mice. Congenitally athymic (nude) mice and their thymus-bearing littermates were produced by mating homozygous (*nu/nu*) males with heterozygous (*nu/+*) females. All mice were of BALB/c background and 6 to 8 weeks old at the start of the study.

Germfree mice were originally derived from NIH BALB/c nude mice by cesarean derivation and have since been bred

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and housed in flexible film isolators at the University of Wisconsin Gnotobiotic Research Laboratory. The microbial status of each mouse was assessed by methods previously described (1, 13).

Colonization of mice with *C. albicans*. Adult GF mice were inoculated (orally) with *C. albicans* by dipping a swab into an inoculum of *C. albicans* (10^6 viable cells per ml), placing it into the mouth of each adult GF mouse, and rotating it 2 to 3 times over the tongue and cheeks. Additionally, adult GF mice became naturally colonized after they were introduced into an isolator and came in contact with *C. albicans*-colonized mice. Under these inoculation conditions, adult GF mice were quickly (24 h) colonized with *C. albicans* in a nontraumatic way. No colonization-associated deaths have ever been observed with athymic or euthymic mice that were colonized with *C. albicans* in this manner.

Enumeration of viable *C. albicans*. Mice were killed by ether inhalation and dissected immediately. The appropriate organs were removed and placed in tissue homogenizers containing 5 ml of sterile PBS. Each organ was homogenized separately, and dilutions were made of each homogenate. The number of viable cells in organ homogenates was determined by plating on Sabouraud dextrose agar. Colonies were counted after 24 h of incubation at 37°C. Data are expressed as the number of CFU per gram (dry weight) of organ. For some studies, tissues were rinsed 2 to 3 times in PBS to wash away intestinal contents.

Histology. Tissues from *C. albicans*-MA mice were excised and immediately placed into Hollande-Bouin fixative. The tissue was embedded in paraffin, sectioned, stained with periodic acid-Schiff, and counterstained with azure A-eosin B or hematoxylin and eosin.

Preparation of cytoplasmic antigens. *C. albicans* was grown on a shaking H_2O bath for 24 h at 37°C in Sabouraud dextrose broth. Yeast cell cultures were centrifuged (500 × *g*) for 10 min, washed twice, and suspended in PBS. Cells were broken to release cytoplasmic antigen by passage through a French pressure cell (SLM/Aminco, Urbana, Ill.) at 10,000 to 15,000 lb/in^2 at 4°C. The broken cells were then centrifuged at 20,000 × *g* for 30 min to separate out cell walls. The cytoplasmic antigen was shown to contain 5 to 10 mg of protein per ml by use of the Pierce BCA protein assay reagent (Pierce Chemical Co., Rockford, Ill.).

Lymphocyte proliferation assay. Mice were sacrificed by cervical dislocation. Spleens were aseptically removed and spleen cell suspensions were prepared in cold RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.). Spleen cells were washed twice in cold RPMI by centrifugation (120 × *g*) for 6 min. The number of viable lymphocytes was determined by trypan blue exclusion. The splenic lymphocytes were suspended in RPMI that was supplemented with bovine calf serum (5%), 2-mercaptoethanol (5×10^{-5} M), HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) (1 mM), glutamine (200 mM), penicillin (20,000 U/ml), and streptomycin (20,000 U/ml). The latter medium is designated RPMI-c. Spleen cells, 2.5×10^5 in 0.2 ml of RPMI-c, were added to each well of a 96-well microtiter plate (Falcon; Becton Dickinson Labware, Lincoln Park, N.J.). Lipopolysaccharide (Difco Laboratories, Detroit, Mich.) was used at concentrations of 10, 1, and 0.1 μg per well; concanavalin A (ConA) (Sigma Chemical Co., St. Louis, Mo.) was used at concentrations of 4, 0.4, and 0.1 μg per well; and phytohemagglutinin (Sigma) was used at concentrations of 10, 5, and 1 μg per well. *Candida* antigen, prepared as described above, was used at concentrations of 50, 25, 10, 2, and 1 μg per well. Control wells had no mitogen or antigen added. All mitogen and antigen concentrations were run in triplicate. Plates were kept in a humidified CO_2 (5%) incubator at 37°C for 72 or 96 h. For the last 18 h of a 72- or 96-h culture period, 0.2 μCi of [^{3}H]thymidine (volume, 25 μl) (Dupont, NEN Research Products, Boston, Mass.) was added to each well. The cultured cells were harvested with an automatic cell collector (Otto Hiller, Madison, Wis.), and incorporated [^{3}H]thymidine was detected in a liquid scintillation counter (Beckman Instruments, Inc., Los Angeles, Calif.).

Delayed-type hypersensitivity skin tests (footpad swelling). Germfree and *C. albicans*-MA mice were given intrafootpad injections of *C. albicans* antigen in 25 μl of saline (125 μg of protein per injection). The right footpad was injected with *Candida* antigen, and the left footpad of each mouse was injected with 25 μl of saline. Footpads were measured with Schnelltaster calipers (H. T. Kröplin, Hessen, Federal Republic of Germany) at 0, 12, 24, and 48 h. Germfree animals whose footpads were injected with either saline or *C. albicans* antigens were used as controls.

Statistics. The significance of these data was analyzed (Student's *t* test and a one-way analysis of variance) by using the Minitab II statistical computing system at the University of Wisconsin Computing Center.

TABLE 1. Viable *C. albicans* in the alimentary tracts of athymic (*nu/nu*) and euthymic (*nu/+*) BALB/c mice after 11 weeks of monoassociation

Mouse genotype and tissue/organ	Amt[a] of *C. albicans* found in:	
	Tissue and contents	Tissue only
nu/nu		
Esophagus	5.9 ± 0.5	3.8 ± 2.0
Stomach	8.7 ± 0.8	8.2 ± 0.9
Small intestine	7.4 ± 0.2	6.6 ± 0.3
Colon	8.3 ± 0.1	7.1 ± 0.3
Cecum	8.7 ± 0.1	6.9 ± 0.7
MLN		4.1 ± 0.4
nu/+		
Esophagus	5.5 ± 0.3	3.5 ± 1.8
Stomach	7.2 ± 0.2	4.8 ± 0.5
Small intestine	6.6 ± 0.2	5.7 ± 0.4
Colon	7.9 ± 0.1	6.7 ± 0.2
Cecum	8.3 ± 0.1	7.6 ± 0.5
MLN		4.3 ± 0.6

[a] Data are expressed as the $\log_{10}$ number of viable *C. albicans* per g (dry weight) plus or minus the standard error of the mean cultured from six mice.

RESULTS

Table 1 shows the number of viable *C. albicans* cultured from mesenteric lymph nodes (MLN) and from different sections of the alimentary tracts of gnotobiotic mice colonized with a pure culture of *C. albicans* for 11 weeks. As previously reported (1), *C. albicans* quickly colonizes and persists in the alimentary tracts of gnotobiotic *nu/nu* and *nu/+* mice in large numbers. Even though viable *C. albicans* was isolated from MLN, no progressive systemic infection occurred in either the *nu/nu* or *nu/+* mice, although sporadic low numbers of *C. albicans* could be isolated from internal organs (1).

Table 2 summarizes microscopic observations made on histological sections taken from the stomachs of mice killed at different time intervals after alimentary tract colonization. By 1 to 2 weeks after colonization, *C. albicans* had invaded mucosal tissues in the stomachs of *nu/nu* and *nu/+* mice.

TABLE 2. Histology of stomach tissue from *C. albicans*-colonized athymic (*nu/nu*) and euthymic (*nu/+*) mice

Time after alimentary tract colonization (wk)	Hyphae observed in mice[a]	
	nu/nu	*nu/+*
1	Yes	Yes
2	Yes	Yes
3	Yes	Yes
5	Yes	Yes
10	Yes	No
15	Yes	No
24	Yes	No

[a] Observed with periodic acid-Schiff stains of the stomach tissues from three mice at each time interval.

This *C. albicans* infection of murine stomachs was only observed at the junction between the secretory and keratinized epithelium (Fig. 1). *nu/nu* and *nu/+* mice appeared to be equally susceptible to the initial infection. By 10 weeks after colonization, it appears (from microscopic observations of stomach sections) that the *nu/+* but not the *nu/nu* mice could clear hyphae from the cardia-atrial junction of their stomachs (Fig. 2). A similar set of events also took place on the dorsal tongue surface, the only other site of the murine alimentary tract in which *C. albicans* hyphae were observed in either *nu/nu* or *nu/+* mice (Fig. 3). The dorsal surface of the tongue remained chronically infected (24 weeks) in the *nu/nu* mice, but the infection was apparently also resolved in *nu/+* mice by week 10. Even though *nu/nu* mice manifest chronic fungal infection of mucosal surfaces (i.e., in the stomach and on the dorsal surface of the tongue), *C. albicans* hyphae were not observed in histologic sections of the duodenum, jejunum, ileum, cecum, and colon.

Lymphocyte proliferation assay. Table 3 shows the blastogenesis response of splenocytes from GF (control) and *C. albicans*-colonized (pure culture) mice.

GF *nu/nu* and *nu/+* mice had only a minimal (background) response to *C. albicans* antigens in the lymphocyte proliferation assay (Table 3). Although colonized and infected with *C. albicans* for up to 20 weeks, *C. albicans*-MA *nu/nu* mice could not mount a lymphocyte proliferative response to *C. albicans* antigen that was greater than background. Conversely, spleen cells from *nu/+* mice showed a positive in vitro lymphocyte proliferative response from 3 to 22 weeks after colonization and infection with *C. albicans*. This blastogenic response was evident in *nu/+* mice that appeared (on the basis of histologic findings) to eliminate hyphal forms of *C. albicans* from infected mucosal sites on the dorsal surface of the tongue and in the cardia-atrial junction in the stomach. Table 3 also demonstrates that athymic mice had only minimal background responses to ConA, a T-cell mitogen, early after colonization but that their responses to ConA increased 17 and 24 weeks after colonization. The blastogenic response to lipopolysaccharide was consistently evident with spleen cells from *nu/nu* and *nu/+* mice (Table 3).

Delayed-type hypersensitivity response after colonization with *C. albicans*. We also tested GF and *C. albicans*-

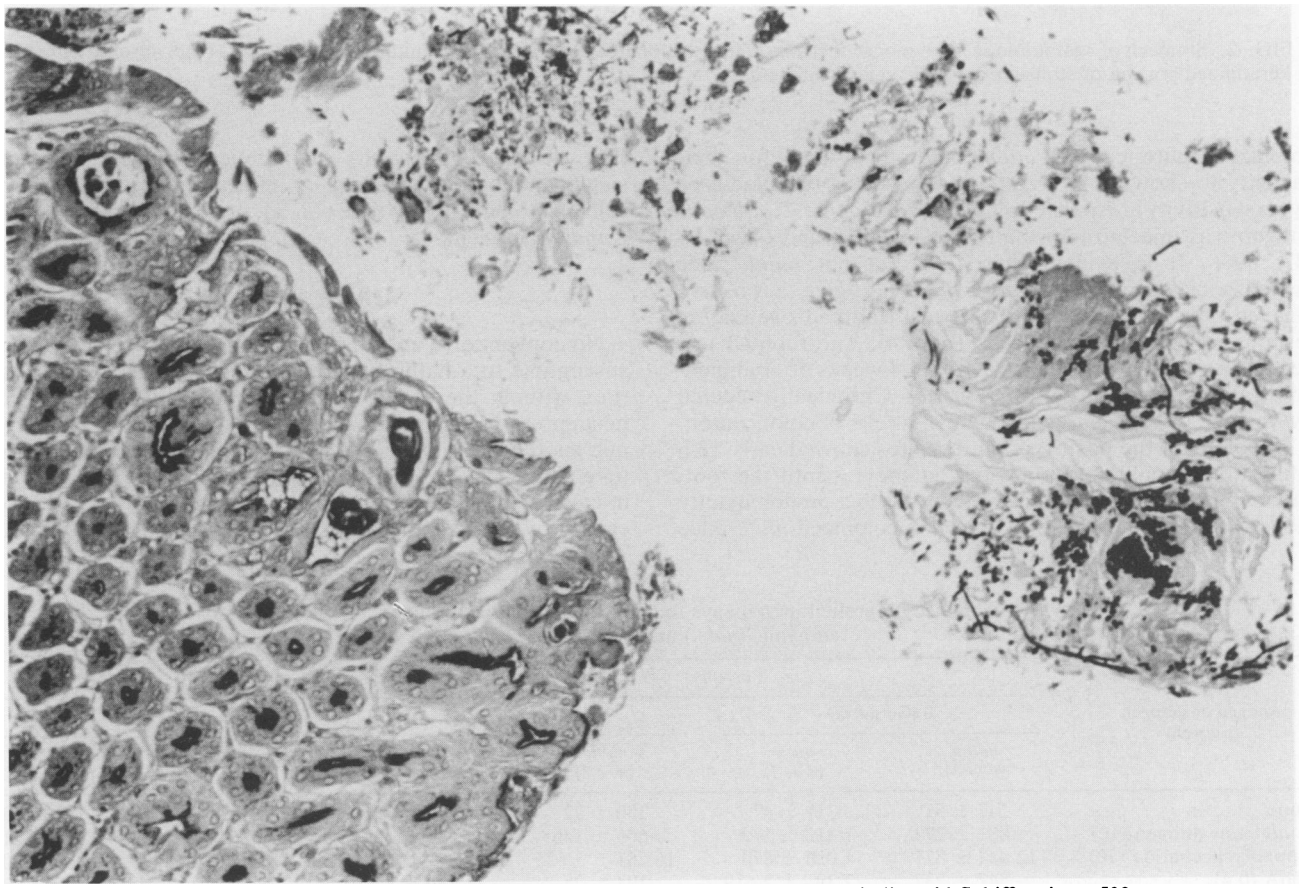

FIG. 1. *C. albicans* hyphae in keratinized tissue of murine stomach. Periodic acid-Schiff stain, ×500.

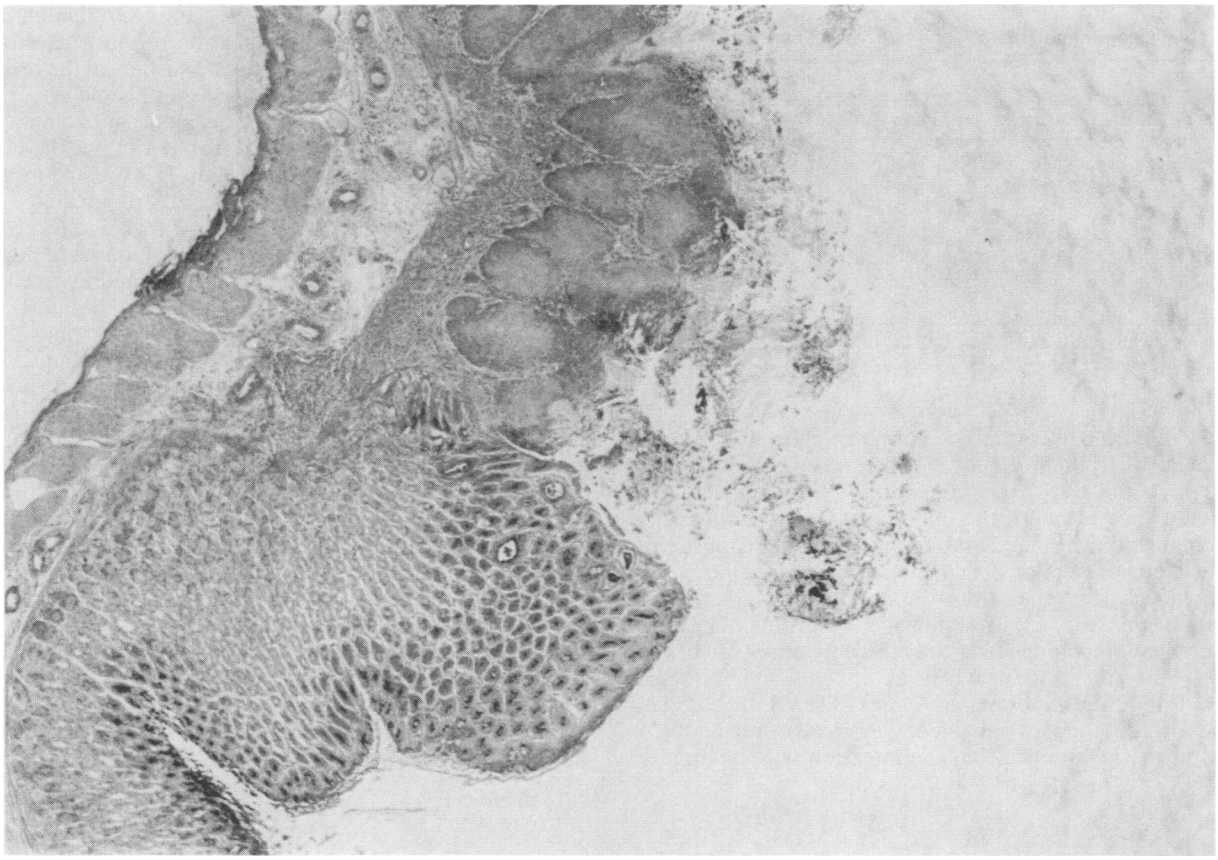

FIG. 2. Stomach of a gnotobiotic *nu*/+ mouse that was colonized for 10 weeks with a pure culture of *C. albicans*. Note absence of hyphae in keratinized portion of stomach. Periodic acid-Schiff stain, ×75.

colonized (pure culture) *nu*/*nu* and *nu*/+ mice for their capacity to show an in vivo footpad swelling (delayed-type hypersensitivity) response to *Candida* antigens. *C. albicans* antigen was injected into the footpads of GF and *Candida*-MA mice. It is evident that *nu*/+ but not *nu*/*nu* mice monoassociated with *C. albicans* could give a positive delayed-type hypersensitivity skin test (footpad swelling) in response to *Candida* antigens (Table 4). The footpad test became positive in *nu*/+ mice at about 4 weeks after alimentary tract colonization with *C. albicans*. Chronically infected *nu*/*nu* mice were negative through 16 weeks of colonization.

Histology of the positive skin test sites showed early 12-h infiltration of polymorphonuclear leukocytes into the footpad; at 24 and 48 h, this had shifted over to a predominantly monocytic infiltrate in the *C. albicans*-colonized *nu*/+ mice (Fig. 4). *C. albicans*-MA *nu*/*nu* mice did not show any significant footpad swelling or infiltration of inflammatory cells (histology) at any time (12, 24, or 48 h) after injection of *Candida* antigens.

DISCUSSION

No conventional animal models to date have proven to be susceptible to a natural mucosal infection with *C. albicans* (i.e., without the use of oral antibiotics, trauma, or immunosuppressive or cytotoxic drugs). The athymic and euthymic gnotobiotic models described in this report for the first time allow studies of the immune responses of hosts during induction and resolution of a primary mucosal infection caused by a pure culture of *C. albicans* in isogeneic, immu-

TABLE 3. In vitro lymphocyte proliferation assays following colonization and infection of athymic (*nu*/*nu*) and euthymic (*nu*/+) mice with *C. albicans*

Antigen or mitogen (μg/well)	Response[a] of mice at wk after alimentary tract colonization					
	0 (Germfree)		1		2[b]	
	nu/*nu* (*n* = 3)	*nu*/+ (*n* = 3)	*nu*/*nu* (*n* = 3)	*nu*/+ (*n* = 3)	*nu*/*nu* (*n* = 3)	*nu*/+ (*n* = 3)
None	147 ± 51	319 ± 47	150 ± 22	227 ± 58	107 ± 17	149 ± 10
C. albicans antigen (10)	881 ± 273	1,312 ± 179	606 ± 146	547 ± 55	350 ± 52	517 ± 213
Lipopolysaccharide (10)	12,444 ± 877	14,028 ± 870	16,281 ± 1,625	18,319 ± 911	7,852 ± 2,468	9,940 ± 301
ConA (0.4)	547 ± 237	27,391 ± 2,619	194 ± 28	27,180 ± 1,170	194 ± 10	12,202 ± 633

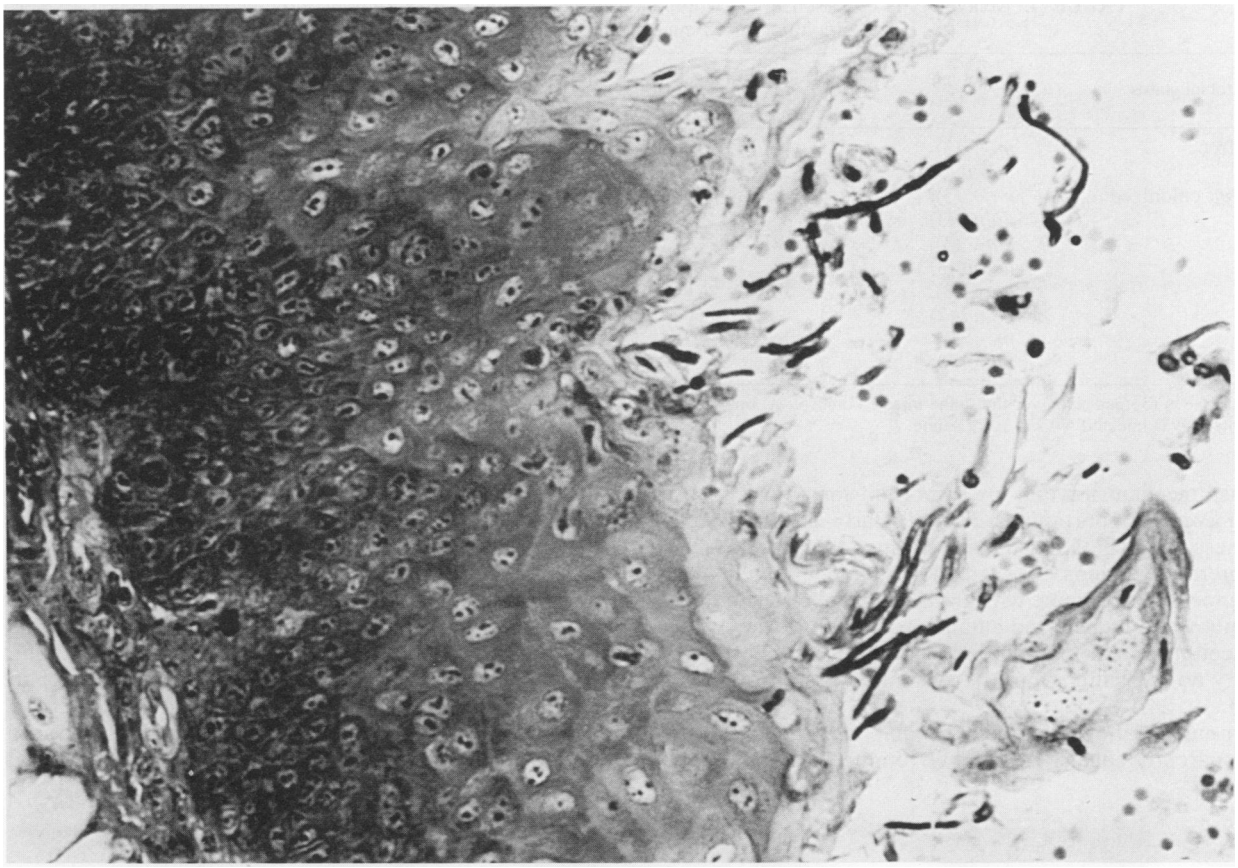

FIG. 3. Dorsal tongue surface of an infected *nu/nu* mouse showing hyphae in keratinized tissue. Periodic acid-Schiff stain, ×1,250.

nocompetent, and congenitally immunodeficient (athymic) mice.

Clinically, patients with mucocutaneous candidosis often have defects in T-cell-mediated immune responses (15). Although mucosal tissues in such patients are often infected with *C. albicans*, the patients do not appear to have problems with systemic candidosis (2, 15, 18).

In order to study innate and acquired immunity to mucosal and systemic candidosis, it is desirable to have an animal model that mimics the disease seen in human patients suffering from candidosis and does not rely on antibiotics, immunosuppressive or cytotoxic agents, or bolus injections of *C. albicans* into neonates or adults for colonization and infection of mucosal surfaces in the alimentary tract. It would be of further benefit to have the yeast chronically colonize the intestinal tract and infect mucosal tissues without causing progressive systemic disease in the animal model. Preferentially, the innate and acquired immune responses of the host should be directed at a pure culture of *C. albicans* and not at the myriad of bacterial species that colonize mucosal surfaces of conventional animals. Pure cultures of *C. albicans* should be used to study the innate and acquired immune responses of congenitally immunodeficient animal models (that mimic immunodeficiencies seen in patients suffering from *Candida* infections) and their immunocompetent counterparts to mucosal and systemic candidosis. Studies of mucosal and systemic candidosis in the latter animal models would identify innate and acquired

TABLE 3—*Continued*

Response[a] of mice at wk after alimentary tract colonization							
3		4		17		22	24
nu/nu (*n* = 3)	*nu/+* (*n* = 3)	*nu/nu* (*n* = 3)	*nu/+* (*n* = 3)	*nu/nu* (*n* = 3)	*nu/+* (*n* = 3)	*nu/nu* (*n* = 3)	*nu/+* (*n* = 3)
142 ± 22	299 ± 16	141 ± 18	352 ± 91	363 ± 51	513 ± 32	540 ± 166	672 ± 234
404 ± 56	2,706 ± 269	520 ± 82	5,176 ± 1,126	993 ± 37	10,897 ± 1,615	9,493 ± 2,554	1,129 ± 254
16,282 ± 1,626	20,003 ± 892	7,659 ± 433	8,342 ± 2,754	19,772 ± 345	21,524 ± 809	21,587 ± 175	20,728 ± 1,779
188 ± 29	21,110 ± 1,226	259 ± 54	11,487 ± 587	3,225 ± 1,148	24,250 ± 970	23,410 ± 1,065	7,113 ± 1,106

[a] Counts per minute of [^{3}H]thymidine incorporated per 2.5 × 10^5 spleen cells. Values represent amounts of labeled thymidine incorporated by spleen cells during the last 17 h of a 96-h incubation at 37°C and 5% CO_2 for *Candida* antigen and during the last 17 h of a 72-h incubation for mitogens (ConA and lipopolysaccharide) plus or minus the standard error of the mean.

TABLE 4. Delayed-type hypersensitivity (footpad swelling) response of gnotobiotic athymic (*nu/nu*) and euthymic (*nu/+*) mice colonized with *C. albicans*

Microbial status	Genotype (no. of mice)	Wk after oral challenge	Increase[a] in footpad swelling (mm ± SE) at hour after antigen injection			
			0	12	24	48
Germfree	*nu/+* (3)		0.03 ± 0.03	0.03 ± 0.03	0	0.07 ± 0.03
	nu/nu (3)		0	0	0.03 ± 0.03	0.07 ± 0.03
Candida colonized	*nu/+* (3)	4	0.03 ± 0.03	0.03 ± 0.03	0.13 ± 0.07	0.30 ± 0.06
	nu/nu (3)		0.03 ± 0.03	0.03 ± 0.03	0	0
	nu/+ (3)	8	0	0.07 ± 0.03	0.23 ± 0.09	0.20 ± 0.00
	nu/nu (3)		0	0.03 ± 0.03	0.07 ± 0.03	0
	nu/+ (3)	14	0.07 ± 0.03	0.50 ± 0.00	0.50 ± 0.12	0.03 ± 0.03
	nu/nu (2)		0.05 ± 0.05	0	0.05 ± 0.05	0.05 ± 0.05
	nu/+ (3)	16	0	0.30 ± 0.17	0.50 ± 0.03	0.40 ± 0
	nu/nu (3)		0	0	0	0

[a] Increase in footpad size over the saline-injected control. At time zero, *C. albicans* antigen (25 μl; 125 μg of protein) was injected into the right footpad. The left footpad was injected with 25 μl of saline.

defense mechanisms that are important for a host to resist this universal opportunistic disease. Early studies designed to assess the role of T cells in resistance to candidosis used parenteral injections to systemically infect conventional, immunocompetent mice with *C. albicans*, even though most patients with mucosal and cutaneous candidosis have defective cell-mediated immune responses and such patients usually do not suffer from the systemic form of the disease (2, 15, 19, 24).

Our gnotobiotic model indicates that *nu/nu* and *nu/+* mice are apparently equally susceptible to mucosal candidosis on the dorsal surface of the tongue and in the cardial section of the stomach (i.e., at the junction of keratinized and secretory epithelial cells). Although chronic MLN candidosis was present in *nu/nu* and *nu/+* mice, progressive systemic infections did not occur in mice of either genotype. The main difference in mucosal candidosis infections of *nu/nu* and *nu/+* mice was that *C. albicans* caused a chronic mucosal infection in *nu/nu* mice, whereas *nu/+* mice apparently cleared hyphae from infected mucosal tissues of the tongue and stomach. *nu/nu* mice also did not appear to be sensitized by *C. albicans* colonization and mucosal infection, since

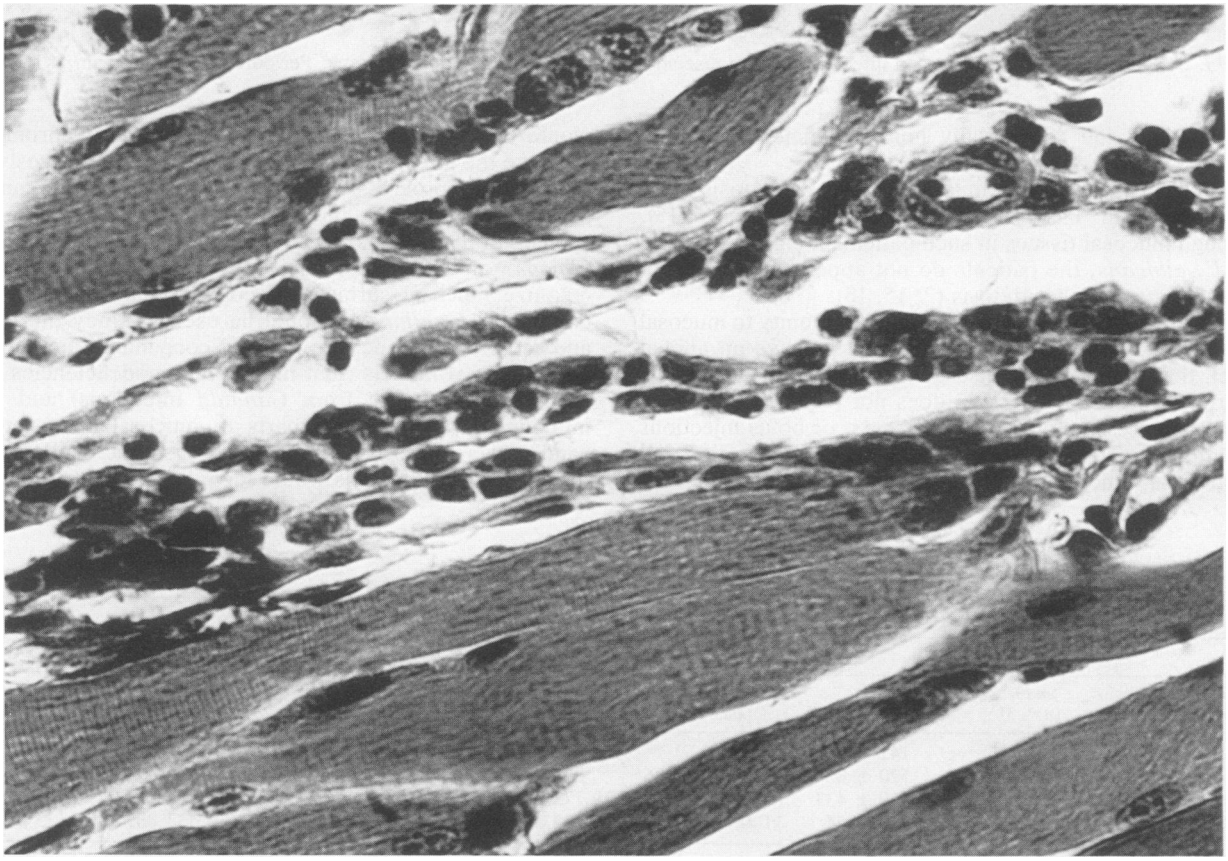

FIG. 4. Monocyte infiltration into the footpad of a *C. albicans*-colonized *nu/+* mouse that was injected with *Candida* antigen (cytoplasmic antigen) 24 h previously. Hematoxylin and eosin, ×1,250.

lymphocyte proliferation assays and an in vivo footpad swelling (delayed-type hypersensitivity) response to *Candida* antigens were not evident in these chronically infected animals lacking functional T cells. Histology showed that a primary *C. albicans* infection at a mucosal surface can apparently be cleared by the *nu*/+ mice which developed in vitro and in vivo correlates of T-cell-mediated immunity for *Candida* antigens after they were colonized and infected. The important immune mechanisms involved in the clearance of the mucosal infections in *nu*/+ mice remain to be clarified, but a murine model for carrying out studies aimed at detailing these important anti-*C. albicans* host defense mechanism(s) is now available. It was also noteworthy that a chronic infection of MLN was evident in both athymic and euthymic mice. The *nu*/+ mice manifested chronic infections of their MLN at times when cell-mediated immune responses were evident. This may indicate that there is a constant translocation of *C. albicans* across the gastrointestinal tracts in these gnotobiotic mice.

Our studies reveal that cell-mediated immune responses take place in *nu*/+ gnotobiotic mice colonized with a pure culture of *C. albicans*. Although mice of both genotypes manifested mucosal infections and chronic infections of their MLN, progressive infections of internal organs did not occur in either *nu*/*nu* or *nu*/+ mice and apparently was not required to sensitize the *nu*/+ mice to *C. albicans* antigens. The *nu*/+ mice appear to be able to resolve the mucosal infections that follow alimentary tract colonization, whereas *nu*/*nu* mice remained chronically infected at two mucosal sites. The *nu*/*nu* and *nu*/+ murine models of candidosis described in this report mimic mucosal candidosis observed in patients with defects in T-cell-mediated immunity (2, 15, 19, 27) and will allow a variety of controlled experiments to be carried out to delineate the role of antibody- and cell-mediated immunity in resistance to mucosal and possibly also systemic candidosis of endogenous origin.

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