

# Inoculation Candidiasis in a Murine Model of Severe Combined Immunodeficiency Syndrome

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To further elucidate the importance of T- and B-lymphocyte-mediated responses in host defense against systemic infection with *Candida albicans*, we studied this infection in a murine model of severe combined immunodeficiency (SCID). The course of inoculation candidiasis in these mice, which lack functional T and B lymphocytes, was compared with that in immunologically normal BALB/c mice. Mice were inoculated intravenously with  $10^5$  yeast cells. Quantitative cultures of liver, spleen, and kidneys were performed with necropsy specimens obtained 1, 3, 7, 10, 14, and 21 days after this intravenous inoculation. The differences in the time courses of recovery of organisms from liver and spleen specimens were not significantly different in the SCID mice compared with the BALB/c mice. The recovery of *C. albicans* from the kidneys was significantly lower in the SCID mice, indicating less persistence of the organism in the kidneys of the SCID mice than in those of the BALB/c mice. These data indicate that defense mechanisms other than T- and B-lymphocyte-mediated mechanisms are primarily responsible for host defense against inoculation candidiasis.

Studies of host defense against infection with *Candida albicans* have demonstrated roles for humoral factors, phagocytic cells, classical cell-mediated immunity, and antibody-producing cells (39, 42). However, their relative contributions in protection against specific forms of candidiasis remain to be elucidated.

A role for classical thymus-dependent cell-mediated immunity in protection against mucosal candidiasis is supported by enhanced susceptibility to this form of candidiasis in athymic mice (17, 39), patients with chronic mucocutaneous candidiasis (23), and patients with acquired immunodeficiency syndrome (44). However, data on the effect of T-lymphocyte-mediated systems in protection against invasive candidiasis do not establish as clear a role (2, 6, 10, 11, 14, 16, 20, 25, 26, 30, 40, 41). Similarly, a role for a protective effect for B-lymphocyte-mediated mechanisms is not clearly established (1, 12, 16, 18, 32, 35, 39). Natural killer cells do not appear to have a major role in resistance to candidal infections (45, 48).

The discovery of a mouse strain with severe combined immunodeficiency (SCID) syndrome (4) provided an opportunity to study the host response to intravenous inoculation of *C. albicans* in the absence of functional T and B cells and thus further characterize the importance of these cells in host defense. We compared the course of inoculation candidiasis in immunologically normal mice with that in mice with SCID syndrome.

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## MATERIALS AND METHODS

**Organism and culture techniques.** All experiments were performed with *C. albicans* type A strain B311 (21). For animal inoculations, organisms were grown in the yeast phase by overnight incubation in Sabouraud glucose broth on an orbital shaker at 37°C, harvested by centrifugation, washed three times in sterile 0.9% NaCl, and counted in a hemacytometer. An inoculum of  $10^5$  yeast cells per 0.1 ml was then prepared in sterile 0.9% NaCl. A sample of the inoculum was quantitatively cultured by standard pour-plate technique in Sabouraud glucose agar.

**Animal models.** C.B-17 SCID mice (SCID mice) were bred in a pathogen-free environment at the Oklahoma Medical Research Foundation. Several days before inoculation they were transferred to microisolator cages (Nalgene Labware Div., Nalge/Sybron Corp., Rochester, N.Y.) in a separate animal facility. Food, water, bedding, and cages for the SCID mice were sterilized before use. The phenotypic purity of the SCID mice was tested by radial immunodiffusion for immunoglobulin M (IgM) (Whittaker Bioproducts, Walkersville, Md.). An undiluted 5- $\mu$ l serum sample obtained from each SCID mouse at necropsy was tested in accordance with kit instructions. Serum from one animal in each group of BALB/c mice was tested as a positive control.

BALB/c mice, the immunoglobulin-heavy-chain congenic partner of the SCID mice (36), were used as the control group. They were also obtained from the Oklahoma Medical Research Foundation. They were housed in ordinary mouse cages.

All mice were 6 to 10 weeks old at the time of inoculation. Equal numbers of male and female mice of each strain were used in each experimental group. The animals were housed in groups of four and given food and water ad libitum.

Animals were inoculated in groups of four SCID and four BALB/c mice. On day zero, all animals received a 0.1-ml lateral tail vein injection containing  $10^5$  yeast cells, as indicated by hemacytometer count. Groups of animals were

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then sacrificed at 1, 3, 7, 10, 14, and 21 days after inoculation.

After induction of general anesthesia, a blood sample was obtained by cardiocentesis, and serum obtained by centrifugation was stored at  $-20^{\circ}\text{C}$  for subsequent testing. An autopsy was then performed, using sterile technique, for harvest of the liver, spleen, and kidneys from each animal. Portions of each of these three organs from one of each group of four animals were fixed in Formalin and prepared for histopathologic examination with hematoxylin-eosin and periodic acid-Schiff stains. The organs were individually homogenized (the two kidneys were homogenized together) in 10 volumes (wt/vol) of normal saline (Omni-Mixer; Omni, Waterbury, Conn.), and serial 10-fold dilutions in normal saline were quantitatively cultured by standard pour-plate technique in Sabouraud dextrose agar. After 48 h of incubation at  $37^{\circ}\text{C}$ , the number of CFU of *C. albicans* was enumerated. Results are expressed as  $\log_{10}$  CFU of *C. albicans* per gram of tissue. Negative growth at the lowest workable dilution, 1:10, was assigned a value of  $1.0 \log_{10}$  CFU/g.

**Statistical analysis.** Using the value of  $1.0 \log_{10}$  CFU/g for negative culture results, we calculated the  $\log_{10}$  mean ( $\pm$  standard error of the mean) CFU per gram for all animals of each strain at each postinoculation interval studied. Because of this arbitrary assignment of value to no-growth results and the heterogeneity of variance encountered, nonparametric techniques were chosen for analysis of data. A nonparametric two-factor (interval postinoculation and mouse strain) analysis of variance (47) was used to compare the mean ranked  $\log_{10}$  CFU of *C. albicans* per gram separately for each of the three organs.

## RESULTS

In the screen for phenotypic purity, we found that all SCID mice had undetectable IgM by radial immunodiffusion, indicating IgM levels  $<150 \mu\text{g/ml}$  (data on file, Whittaker Bioproducts). The mean ( $\pm$  standard deviation) IgM level for BALB/c mice has been reported as  $320 \mu\text{g/ml}$  ( $\pm 36 \mu\text{g/ml}$ ) (33). Thus, the IgM levels in our SCID mice were greater than 3 standard deviations below the mean normal levels.

By quantitative culture, the mean inoculum for all mice was determined to be  $(1.09 \pm 0.07) \times 10^5$  CFU of *C. albicans* and ranged from  $3.5 \times 10^4$  to  $2.9 \times 10^5$  CFU.

Neither microabscesses nor microorganisms were detected histopathologically in any of the analyzed specimens from either BALB/c or SCID mice. There was no difference between BALB/c and SCID mice in the gross or microscopic pathology of livers and kidneys. However, the spleens from the SCID mice were small and atrophic, with marked fibrosis and paucity of lymphoid follicles, as previously described (5).

Recovery of *C. albicans* from the livers of the two strains of mice at various intervals postinoculation (Fig. 1A) followed a biphasic pattern, declining until day 7 postinoculation, then increasing until day 14, and then declining again. This pattern of visceral recovery of *C. albicans* was obtained with both mouse strains and has been previously reported (26). By analysis of variance, the difference between mean  $\log_{10}$  CFU per gram recovered at the various postinoculation intervals was significant ( $P < 0.0001$ ). The difference in the recovery of organisms due to mouse strain effect was not significant ( $P = 0.41$ ); that is, we were unable to demonstrate a significant difference between BALB/c and SCID mice in the number of yeast cells recovered from the liver. Also, the

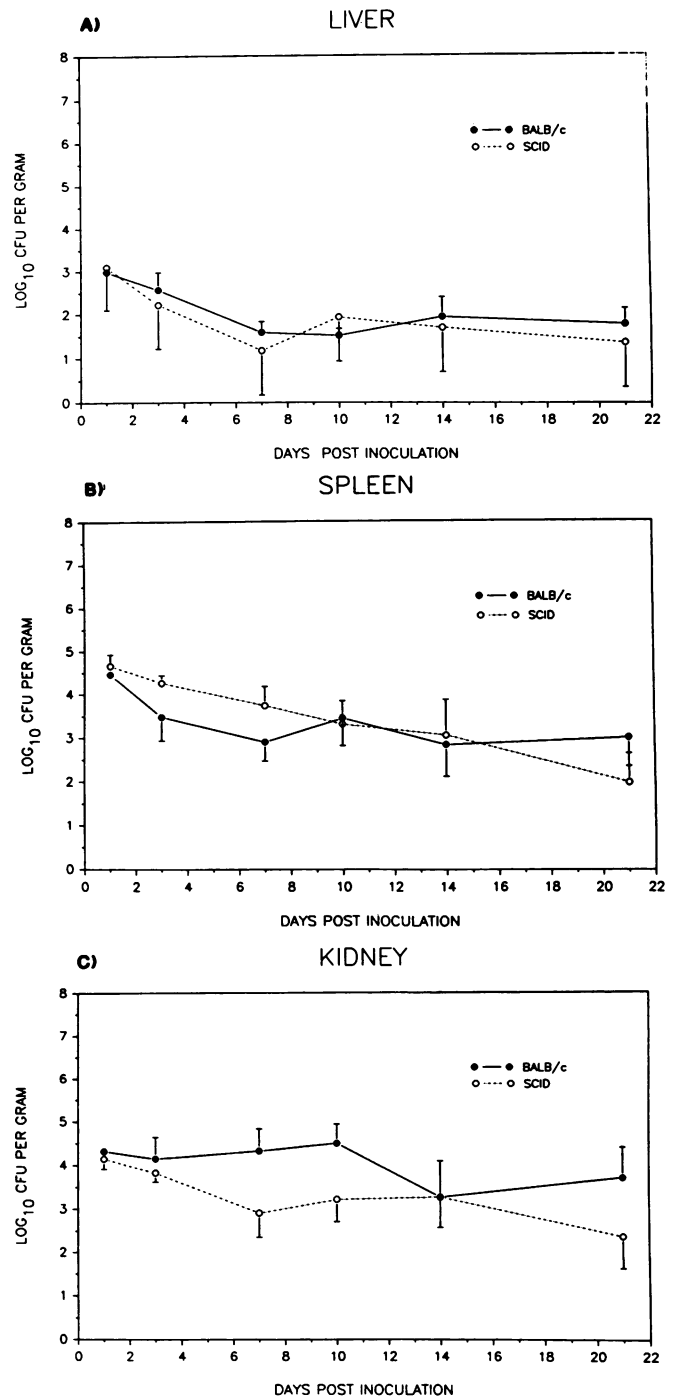


FIG. 1. Recovery of *C. albicans* from livers (A), spleens (B), and kidneys (C) of BALB/c and C.B-17 SCID mice. Points and error bars represent the mean  $\log_{10}$  CFU per gram of tissue and the corresponding standard errors for eight mice inoculated with  $10^5$  live yeast cells on day 0. No growth at the lowest dilution (1:10) was assigned a value of 1.00.

interaction of these two factors (postinoculation interval and mouse strain) was not significant ( $P = 0.91$ ).

The recovery of *C. albicans* from the spleens of the two strains of mice is shown in Fig. 1B. A biphasic pattern was again seen in the BALB/c mice but was less marked in the

SCID mice. The recovery of organisms from spleens was generally higher than the recovery from livers. As with the livers, there was a significant effect on the recovery of organisms from spleens due to postinoculation interval ( $P = 0.004$ ) but no significant effect due to mouse strain (BALB/c versus SCID) ( $P = 0.69$ ) or the interaction of these two factors ( $P = 0.58$ ).

The recovery of *C. albicans* from the kidneys of the two mouse strains at the various postinoculation intervals is shown in Fig. 1C. A slightly biphasic pattern was seen in both mouse strains, but overall the curves were less steep in both species than those for livers or spleens, indicating persistence of more nearly equal numbers of *C. albicans* in the kidneys of both species over the course of the study period. This observation was confirmed by analysis of variance: the effect of postinoculation interval on recovery of *C. albicans* from the kidneys was not significant ( $P = 0.59$ ). The interaction effect (postinoculation interval and mouse strain) was also not significant ( $P = 0.67$ ).

However, unique to the kidneys, there was a significant difference in mean  $\log_{10}$  CFU per gram recovered between the two mouse strains ( $P = 0.007$ ). The recovery of *C. albicans* from the kidneys of BALB/c mice was higher than from the kidneys of SCID mice. For all postinoculation intervals, the mean  $\log_{10}$  recovery from the kidneys of BALB/c mice was  $4.05 (\pm 0.22)$  CFU/g, while that from the SCID mice was  $3.29 (\pm 0.22)$  CFU/g. In our nonparametric analysis of variance, the mean ranked recovery from the kidneys of BALB/c mice was 56.14, compared with 40.85 from the kidneys of SCID mice.

## DISCUSSION

The SCID mutant strain was derived from a C.B-17 inbred strain of BALB/c mice (4). SCID mice have markedly reduced or absent mature T and B cells as a result of defects in rearrangement of immunoglobulin and T-cell antigen receptor genes (15, 19, 24). Furthermore, intrinsically defective B-lineage lymphocytes from SCID mice can be propagated in culture (46). SCID mice have normal numbers of myeloid and erythroid cells, which differentiate normally (5). This strain appears to have an expanded pool of natural killer cells which respond normally in *in vitro* functional assays (24). Macrophages are present in normal numbers and respond normally to T-cell-independent stimuli (3).

Our study of the course of inoculation candidiasis revealed no significant differences in the recovery of *C. albicans* from livers and spleens of SCID and BALB/c mice. SCID mice had lower numbers of organisms recovered from the kidneys, but it seems prudent not to overemphasize the difference in one organ alone. It is apparent from our data however, that SCID mice, despite the lack of functional lymphocytes, did not have enhanced susceptibility to *C. albicans* after intravenous inoculation.

The report that normal mice, but not thymectomized, irradiated, and reconstituted mice, acquire increased resistance to *C. albicans* if they are vaccinated by cutaneous infection with live *Candida* cells suggests that T cells can confer protection against systemic candidiasis (14, 30). Similarly, long-term colonization of mice has been reported to produce T-cell-mediated protection against inoculation candidiasis (11). In contrast T-cell-deficient nude mice have been found relatively more resistant to infection with this organism than immunologically normal controls (2, 6, 14, 16, 25, 40, 41), perhaps because of increased macrophage acti-

vation in these animals (39). The first difference between SCID mice and nude mice is that SCID mice, in addition to not having functional T cells, possess no active B cells (5). Another important difference between SCID mice and other experimental T-cell-deficient mice is that macrophages in SCID mice have not been found to be in an activated state or increased in number (3). Our data thus extend previous studies on T-cell-depleted mice, in that SCID mice, without functional T cells, were not more susceptible to inoculation candidiasis.

Although the role of B cells and antibodies in host defense against candidiasis has been studied extensively (1, 12, 14, 18, 32, 35, 38, 39), clear understanding of their relative importance has not emerged (7, 37, 39). Some studies have demonstrated that passive transfer of immune serum can confer resistance to *C. albicans* experimentally in animals and humans (1, 18, 32). A recent study has suggested that B cells and their products may play a role in killing of *C. albicans* *in vitro* in the presence of T cells and macrophages (34). In guinea pigs, antibody-mediated mechanisms of immunity have not been found to be operative in defense against parenteral challenge with *C. albicans* (43). In our SCID mice, the additional absence of B cells did not affect the course of infection in the tissues examined, suggesting that this subset of lymphocytes may not be critical in defense against this type of *C. albicans* infection.

Even if one did not expect B and T lymphocytes to be involved in clearance of *C. albicans* in the first few days following primary intravenous inoculation, one might expect a significant involvement later in the course of infection (after 10 to 14 days). Even at these times, however, we found no difference in the recovery of organisms from livers and spleens. Thus, even at times when acquired immunity is operative, we could not demonstrate a role for T or B lymphocytes in host defense against this form of candidiasis. This suggests that lymphocyte-independent mechanisms, which are sufficient in SCID mice, are able to control this infection.

A serum factor has been described which exerts a protective effect by causing yeast clumping (29). Human serum is not directly candidacidal, but the organism activates the alternative complement pathway, thus generating chemotactic factors (13, 31). *In vitro* studies have demonstrated that macrophages and polymorphonuclear leukocytes are capable of ingesting and killing *C. albicans* hyphae and yeast cells (8, 9, 27). The role of phagocytic cells in host defense is evidenced further by the observations that invasive candidiasis occurs most frequently in patients with quantitative deficiencies of phagocytic cells (7) and that animal models and patients with isolated neutrophil functional defects are more susceptible to invasive candidiasis (12, 22, 28). Further studies to delineate the mechanisms which are critical in defense against intravenously inoculated *C. albicans* should focus on complement and other serum factors, macrophages, and polymorphonuclear leukocytes.

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