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# Systemic Candidosis in Silica-Treated Athymic and Euthymic Mice

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Intravenous silica injections were used to assess the role of macrophages in the resistance of BALB/c nude and euthymic mice to systemic candidosis. CFU of *Candida albicans* in the kidneys, livers, and spleens of saline- or silica-treated mice were enumerated at various times after inoculation with $10^4$ viable yeast cells. The number of *C. albicans* organisms recovered from the kidneys of silica-treated euthymic mice was similar to the number recovered from saline-treated controls during the first 3 days of infection; however, at every assay period thereafter, the number of organisms recovered from the kidneys of silica-treated mice was dramatically reduced (100- to 1,000-fold). Conversely, silica-treated nude mice were no more susceptible to systemic candidosis than were saline-injected nude mice. Silica treatment did not alter the ability of treated or control mice to clear *C. albicans* from the liver and spleen. These results demonstrate that macrophages play an important role in susceptibility to *Candida* infections.

Candidosis (candidiasis) is a general term describing a variety of clinical conditions, all of which are caused by members of the genus *Candida* and most by the species *Candida albicans*. Chronic mucocutaneous candidosis, a unique form of the disease, is often associated with T-cell deficiencies or with combined T-cell and B-cell defects (10, 20, 21, 28, 30). Although patients with this form of disease rarely, if ever, suffer from disseminated candidosis, the defense mechanism of greatest importance against *C. albicans* is commonly thought to be T-cell-mediated immunity (20, 21). Thus, a distinction between resistance to mucocutaneous infection with *C. albicans* and resistance to disseminated candidosis is not often made. Because candidosis includes a spectrum of diseases ranging from localized, self-limiting lesions of skin and mucous membranes to a severe and often fatal systemic disease (11, 20), it is to be expected that a variety of host immune mechanisms might interact in response to *C. albicans* infections. The roles the various mechanisms might play and their contributions to overall resistance to *C. albicans* infections are, for the most part, not well defined.

The role of T-cell-mediated immunity in protection against the disseminated form of candidosis is not obvious. In fact, innate or natural immune mechanisms appear to be most important in resistance to disseminated *C. albicans* infection. Cutler (4) has shown that congenitally athymic nude mice are more resistant to parenteral infection with *C. albicans* than are their phenotypically normal euthymic littermates. In addition, Rogers et al. (25) demonstrated that thymus reconstitution of nude mice abrogated this increased resistance. We have demonstrated that germfree mice (nude and euthymic) are also resistant to disseminated, as well as mucocutaneous, candidosis (12, 13). Because many functional activities of macrophages from germfree mice are impaired (7, 8, 17), we proposed that normally functioning macrophages might, in fact, be involved in the pathogenesis of disseminated *Candida* infections.

The studies described in this report were initiated to provide more direct evidence of the role of macrophages in the pathogenesis of disseminated candidosis. We report here that intravenous injection of silica, which is selectively toxic for macrophages (14), decreases the susceptibility of thymus-bearing mice to renal candidosis but does not influence the innate resistance of nude mice to systemic disease.

## MATERIALS AND METHODS

**Microorganism.** *C. albicans* B311 (type A), originally obtained from H. F. Hasenclever, National Institutes of Health, Bethesda, Md., has been maintained in our laboratory by monthly transfers on Sabouraud dextrose agar (SDA) slants. Yeast cells were cultured for 24 h at 3°C on SDA and then stored at 4°C. For experiments, a single stock was prepared with organisms that were grown on SDA for 24 h at 37°C, washed

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from the slants, and stored at −70°C in 1 to 2 ml of Sabouraud dextrose broth at a concentration of $10^8$ viable units per ml. The intravenously (i.v.) administered 50% lethal dose (estimated 28 days post-inoculation) of this strain of *C. albicans* was determined to be $2.0 \times 10^4$ viable units for nude mice and $1.6 \times 10^4$ viable units for their heterozygous littermates. The standard i.v. challenge used in this study was $10^4$ viable units (ca. 0.5 50% lethal dose) in 0.25 ml of phosphate-buffered saline (PBS) injected into the lateral tail vein.

The morphology and biochemical characteristics of *C. albicans* were verified by microscopic observations, colonial morphology on SDA, formation of germ tubes in serum, formation of chlamydospores on chlamydospore agar, and sugar fermentation reactions.

**Mice.** Congenitally athymic (nude) mice and their thymus-bearing phenotypically normal littermates (NLM) were produced by mating homozygous (nu/nu) males to heterozygous (nu/+) females. All mice were backcrossed into the BALB/c strain. Within a given experiment, mice were matched according to age and sex and were 40 to 60 days old at the time of challenge with *C. albicans*.

**Silica.** Silica (Min-U-Sil no. 216; Whitaker, Clarke, and Daniels, Plainfield, N.J.) was a gift from J. L. Krahenbuhl (U.S. Public Health Service Hospital, San Francisco, Calif.). The procedure of preparation of silica for i.v. injection followed previously described methods (19). Briefly, silica dust was suspended in PBS at a concentration of 12 mg/ml and autoclaved. After brief sonication, 0.25 ml was injected into the tail vein of each mouse. The mice were challenged (i.v.) with *C. albicans* 1 or 4 days after silica treatment.

To substantiate the fact that our treatment protocol results in an impairment of normal macrophage function, we inoculated silica-treated and PBS-treated mice with *Listeria monocytogenes*. The 50% lethal dose (estimated 7 days post-inoculation [23]) for silica-treated mice ($1.0 \times 10^3$) was dramatically reduced as compared with the 50% lethal dose for control mice ($7.9 \times 10^4$).

**Enumeration of *C. albicans* viable units in organs.** Mice were killed by cervical dislocation and dissected immediately. The appropriate organs were removed and placed in tissue homogenizers containing 5 ml of sterile Sabouraud dextrose broth. Each organ was homogenized separately, and dilutions of each homogenate were made. The numbers of viable units in the homogenates were determined by plating the homogenates on SDA. Colonies were counted after 24 and 48 h of incubation at 35°C. The viable units were expressed as the number of CFU per gram of organ.

## RESULTS

**Effect of silica injection on renal candidosis in thymus-bearing NLM mice.** Mice were inoculated i.v. with $10^4$ viable units of *C. albicans* 24 h after an i.v. injection of silica or PBS. At various times thereafter, the mice were killed, and the spleens, livers, and kidneys were removed and homogenized for an enumeration of viable units of *C. albicans*. No infection-induced deaths were evident throughout the assay.

The results (Fig. 1) demonstrate that pretreatment of mice with silica 1 day before inoculation with *C. albicans* dramatically affects the animal's ability to resist renal candidosis. The number of CFU of *C. albicans* recovered from the kidneys of PBS-treated mice increased for the first 5 to 7 days and then remained relatively constant for the remainder of the assay, at approximately 100-fold the original number of units injected. Although there was no apparent difference in the number of CFU cultured from the kidneys of silica-treated and PBS-treated mice during the first 3 days after inoculation, at every subsequent assay period, the number of CFU cultured from the kidneys of silica-treated mice was substantially reduced. As early as 5 days postinfection, the number of CFU recovered from the kidneys of silica-treated mice was at least 100-fold less than the number of CFU from their PBS-treated counterparts. This difference reached a maximum of nearly 10,000-fold by day 11 postinfection, at which time the number of CFU cultured from silica-treated mice was less than the original inoculum.

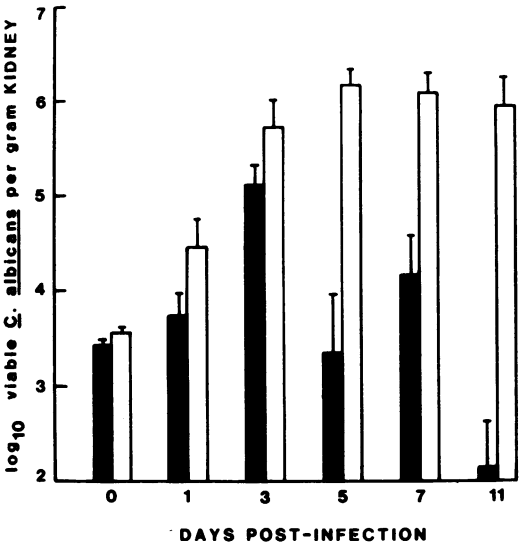


FIG. 1. Recovery of viable *C. albicans* from the kidneys of NLM mice which were injected (i.v.) with 3 mg of silica or PBS 1 day before i.v. challenge with $10^4$ viable units of *C. albicans*. Solid bars, silica-treated mice; open bars, PBS-treated mice. Values represent the means ± standard error of 8 to 18 mice per group.

Because we observed no significant differences in the number of CFU in the kidneys during the first 3 days of infection, it appeared that the effect of silica on renal candidosis might require at least 3 days to manifest itself. To investigate this possibility, we treated mice with silica and then waited 4 days before inoculating them with viable *C. albicans*. The results (Fig. 2)

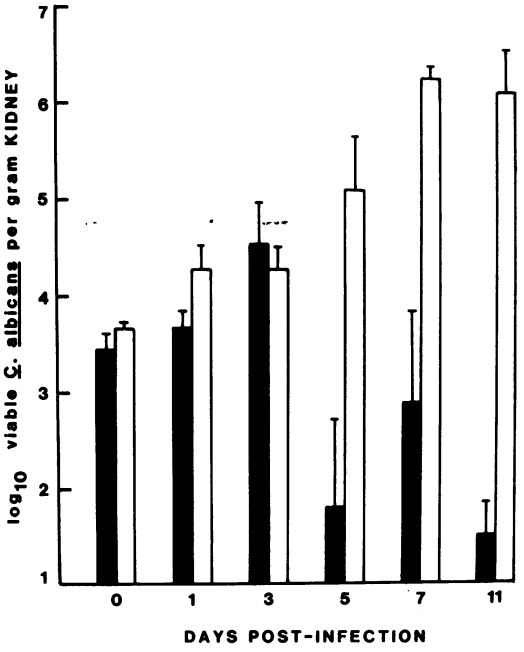


FIG. 2. Recovery of viable *C. albicans* from the kidneys of NLM mice which were injected (i.v.) with 3 mg of silica or PBS 4 days before i.v. challenge with $10^4$ viable units of *C. albicans*. Solid bars, silica-treated mice; open bars, PBS-treated mice. Values represent the means ± standard error of five to six mice per group.

demonstrate that extending the time interval between silica treatment and *Candida* challenge does not appreciably alter the profile of *Candida* clearance from the kidneys of these mice (compare Fig. 1 and 2). As was evident with a 1-day silica pretreatment, no difference in the number of CFU in the kidneys was detected during the first 3 days after *Candida* inoculation. Thereafter, the number of CFU cultured from the kidneys of silica-treated mice was substantially reduced (>1,000-fold).

**Effect of silica injection on renal candidosis in nude mice.** To investigate the effects of silica on renal candidosis in T-cell-deficient mice, congenitally athymic nude mice were injected with 3 mg of silica; 1 day later, they were inoculated with $10^4$ viable units of *C. albicans*. It is noteworthy that the nude mice used in this study were able to tolerate an i.v. injection of 3 mg of silica as well as did their phenotypically normal euthymic littermates.

The results of the enumeration of viable *C. albicans* CFU in the kidneys of nude mice are presented in Fig. 3. We were unable to demonstrate any apparent difference in the number of CFU cultured from silica-treated and PBS-treated nude mice at any period throughout the assay. In contrast to euthymic mice, the number of CFU cultured from the kidneys of nude mice did not appreciably increase with time after challenge (compare Fig. 1 and 3), substantiating the conclusion that nude mice are innately more resistant to renal candidosis.

**Effect of silica injection on *Candida* clearance from the livers and spleens of nude and NLM mice.** The results from the enumeration of *Candida* CFU in the livers and spleens of all of the i.v. challenged mice are presented in Fig. 4. Regardless of pretreatment or immunological (i.e., athymic or euthymic) status, all mice used in this study appeared to be capable of clearing *C. albicans* from their livers and spleens in a similar manner. Less than 1% of the original inoculum remained in these organs at the termination of the experiments.

## DISCUSSION

Macrophages play an important and often crucial role in host immune and nonimmune responses to infectious microorganisms. Silica, an agent which selectively alters macrophage functions in vivo and in vitro (1, 9, 31), has been used to evaluate the role of macrophages in resistance to viruses (6, 33, 34), bacteria (2, 19), and parasites (10). To explore the contribution of macrophages in resistance to i.v. injected *C. albicans*, macrophage activity was disrupted in both euthymic and athymic mice by i.v. injection of silica. The results presented herein dem-

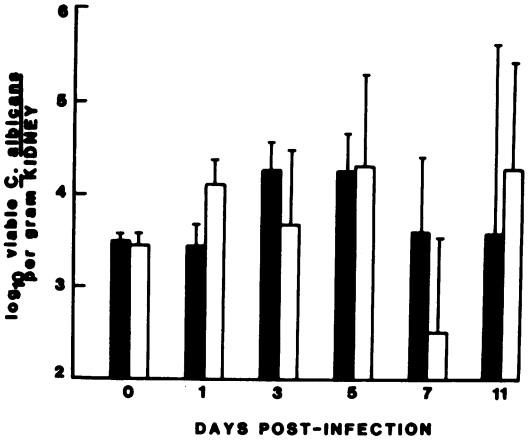


FIG. 3. Recovery of viable *C. albicans* from the kidneys of nude mice which were injected (i.v.) with 3 mg of silica or PBS 1 day before i.v. challenge with $10^4$ viable units of *C. albicans*. Solid bars, silica-treated mice; open bars, PBS-treated mice. Values represent the means ± standard error of four to eight mice per group.

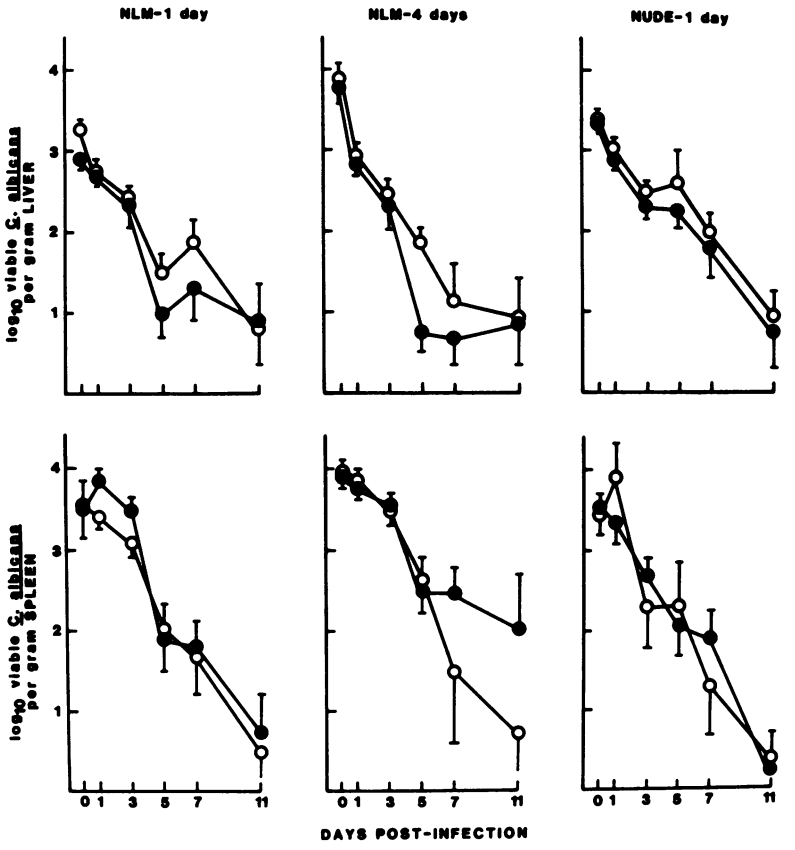


FIG. 4. Recovery of viable *C. albicans* from the livers and spleens of mice which were injected with silica or PBS before i.v. challenge with $10^4$ viable units of *C. albicans*. ●, Silica-treated mice; ○, PBS-treated mice. NLM-1 day, euthymic mice injected with 3 mg of silica or PBS 1 day before *Candida* challenge; NLM-4 days, euthymic mice injected with 3 mg of silica or PBS 4 days before *Candida* challenge; NUDE-1 day, athymic mice injected with 3 mg of silica or PBS 1 day before *Candida* challenge.

onstrate that normally functioning macrophages play a crucial role in *Candida* infections, but not through a direct effector mechanism.

We have shown that pretreatment of normal euthymic mice with silica renders them more resistant than PBS-treated mice to renal candidosis. These results support our original hypothesis that macrophages might be involved in the pathogenesis of disseminated *Candida* infections, and it is tempting to conclude that normally functioning macrophages might engulf but not kill the invading yeast, thereby rendering them protected from other defense mechanisms. However, in light of our findings that increased resistance was not evident in silica-treated NLM mice until after 3 days postinfection and that similar profiles of clearance were evident when silica was administered 1 day or even 4 days before *C. albicans* inoculation, it seems unlikely that this simple explanation can account for the observed resistance. Also, we have shown that similar silica treatment of nude mice does not affect their already high level of resistance to acute renal candidosis.

It has been demonstrated that nude mice possess activated macrophages (3, 18), and it was proposed that this cell type is responsible for the increased resistance of nude mice to candidosis (25). In accord with this hypothesis, Cutler and Poor have shown that macrophage-rich populations of cells from nude mice are candidacidal in diffusion chambers in vivo, but such cells from normal mice are merely candidastatic (5). However, attempts to demonstrate increased resistance in vivo to *C. albicans* by nonspecifically activating macrophages in normal animals have failed (5, 24). In light of our present findings that silica treatment does not affect the outcome of systemic candidosis in nude mice, it seems unlikely that activated macrophages are responsible for resistance to candidosis. Alternatively, our results might demon-

strate that activated macrophages actually are effector cells for *Candida* killing, but that such cells are less susceptible to the effects of silica treatment, or that normal macrophages are activated after silica treatment and escape the toxic effects of silica.

No unequivocal evidence exists that silica has a direct depressive effect on cells other than macrophages, yet silica not only destroys macrophage-specific host activities, but silica treatment also subverts other macrophage-dependent host immune functions. Phagocytosis and antigen processing, antibody production in response to immunization, response of T-lymphocytes to mitogen stimulation, lymphocyte-mediated cytotoxicity, and prolongation of allograft rejection are among the immune functions that have been reported to be influenced by silica treatment (14–16, 22, 26).

The exact mechanism by which silica exerts its effect has not been elucidated. It is possible that subversion of macrophage-dependent immune functions by silica is the result of release of lymphocyte-stimulating monokines (29). In this regard, Sen et al. have demonstrated that pretreatment of mice with a dialyzable extract derived from human leukocytes can lessen the severity of *Candida* infections (27). Also, Vogel et al. have recently reported that the administration of silica to mice potentiate their in vivo sensitivity to bacterial lipopolysaccharide (32). These investigators demonstrated that lipopolysaccharide sensitivity was accompanied by an enhanced production of interferon in vivo in response to lipopolysaccharide stimulation. In addition, it was shown that macrophages from silica-treated mice produced more Interleukin 1 when stimulated in vitro with lipopolysaccharide.

Our results are consistent with the hypothesis that silica pretreatment results in increased production or release of monokines. Because the time interval between silica treatment (1 versus 4 days) and *Candida* inoculation did not affect the profile of *C. albicans* clearance, it appears that activation of host immune functions occurs after specific interaction with the microorganism. Nude mice exhibited neither increased nor decreased susceptibility to *Candida* infection after silica treatment; therefore, it appears that the silica-induced resistance that is evident in NLM mice is mediated through functionally active T-cells.

Although this silica-induced phenomenon requires further investigation, we speculate that monokines liberated from silica-affected macrophages interact with subpopulations of T-cells which in turn stimulate the eventual effector cell after specific interaction with *C. albicans*. Alternatively, the presence of these monokines might result in the removal of T-cell-mediated suppression of the effector population.

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