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Effects of Systemic Cell-Mediated Immunity on Vaginal Candidiasis in Mice Resistant and Susceptible to *Candida albicans* Infections

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Studies to date with CBA/J mice suggest a limited role for systemic cell-mediated immunity (CMI) against vaginal *Candida albicans* infections. The results of the present study show that preinduced *Candida*-specific systemic CMI was equally nonprotective against *C. albicans* vaginal infections in mice with high (BALB/cJ), low (DBA/2), or intermediate (CBA/J) resistance to *C. albicans* infections. Similarly, the locally acquired partial protection against a second *C. albicans* vaginal infection was equally observed with BALB/cJ, DBA/2, and CBA/J mice. These results indicate that observations made previously with CBA/J mice were not murine strain specific and provide additional support for the hypothesis that systemic CMI does not represent a dominant host defense mechanism at the vaginal mucosa.

Recurrent vulvovaginal candidiasis (RVVC) is an opportunistic mucosal infection caused by *Candida albicans*, the etiology of which is poorly understood (14). The high incidence of mucosal candidiasis in patients with reduced cell-mediated immunity (CMI) (i.e., AIDS [11, 13], transplantation [1], and corticosteroid therapy patients [12]) strongly suggests that deficiencies in CMI may similarly play a role in RVVC. However, results of some clinical studies of women with RVVC indicate that systemic CMI is normal (7, 8, 15) and that previously observed reductions in peripheral blood lymphocyte responsiveness (10, 16, 17) may be the result of infection rather than the cause. In contrast to previous hypotheses, we have postulated that deficiencies in local vaginal rather than systemic immune defenses predispose women to RVVC.

Our laboratory has been interested in identifying host defense factors which are important for protection against vaginal *C. albicans* infections. In addition to clinical studies, we have employed an estrogen-dependent murine model of vaginal candidiasis with CBA/J ($H\text{-}2^k$) mice, which are considered neither overly resistant nor susceptible to *C. albicans* infections (9). Under conditions of pseudoestrus, vaginal *C. albicans* infections in CBA/J mice can persist for up to 10 weeks (3). Our first series of studies showed that although a persistent vaginal infection develops in vaginally inoculated mice under conditions of pseudoestrus (3), *Candida*-specific Th1-type CMI is equally induced in the presence or absence of exogenous estrogen (4) and is indistinguishable from that induced by systemic immunization with *C. albicans* culture filtrate antigen(s) (CaCF) (3). Surprisingly, however, the preinduction of systemic *Candida*-specific Th1-type CMI by CaCF did not protect mice against a vaginal *C. albicans* infection, nor did suppression of *Candida*-specific systemic CMI enhance vaginal *C. albicans* burden during the infection period (5). Since the lack of a protective effect in immunized mice could have been due to the inability of soluble *Candida* antigens to adequately induce immunoprotective CMI, subsequent studies evaluated the protective effect of systemic CMI induced by whole *C. albicans* present during a short-lived vaginal infection initiated in the absence of exogenous estrogen (4). Results showed that following resolution of the primary vaginal infection, a second vaginal inoculation under conditions of pseudoestrus resulted in anamnestic-like delayed-type hypersensitivity (DTH) and reduced vaginal fungal burden compared with that in the once-infected (primary-infected) control mice (2). However, this partial protection was retained when *Candida*-specific systemic DTH was suppressed antigen specifically (2) by suppressor T cells induced by intravenous administration of soluble *Candida* antigens (5). Furthermore, in vivo depletion of circulating CD4 and/or CD8 cells had no effect on the natural history of a primary or second vaginal *C. albicans* infection (6). Taken together, these data suggest that local rather than systemic host defense mechanisms provide immune protection at the vaginal mucosa and that a locally acquired immune response was induced by a primary vaginal infection.

Despite the strong evidence for the lack of effect of systemic CMI against *C. albicans* vaginal infections, it was also possible that the observations made with CBA/J mice were host strain specific. Therefore, we were interested in whether similar results would occur with mice with differential susceptibilities to *C. albicans* infections. For this, we performed experiments to analyze the protective role of systemic CMI against *C. albicans* vaginal infections in mice considered more resistant (BALB/cJ, $H\text{-}2^d$) and sensitive (DBA/2, $H\text{-}2^d$) to systemic *C. albicans* infections than CBA/J mice (9). While it is unknown what components are directly responsible for the differential susceptibilities of these mice, it is presumed that acquired and/or innate immune responsiveness is affected by several factors, including complement deficiencies and the class II region of the major histocompatibility complex.

Effects of preinduced *Candida*-specific DTH on experimental vaginitis in murine strains with differential susceptibilities to systemic *C. albicans* infection. CBA/J, BALB/cJ, and DBA/2 female mice (Jackson Laboratory, Bar Harbor, Maine) were immunized with CaCF (10× concentrated supernatants from a blastoconidium culture shaken for 3 days at 25°C in medium from a <12,000-molecular-weight exclusion dialysate preparation [3]) in complete Freund's adjuvant (CFA) (CaCF-CFA) by the subcutaneous injection of 0.1 ml at each of two sites at the base of the tail (3). Control groups of mice received subcutaneous injections of phosphate-buffered saline (PBS) in

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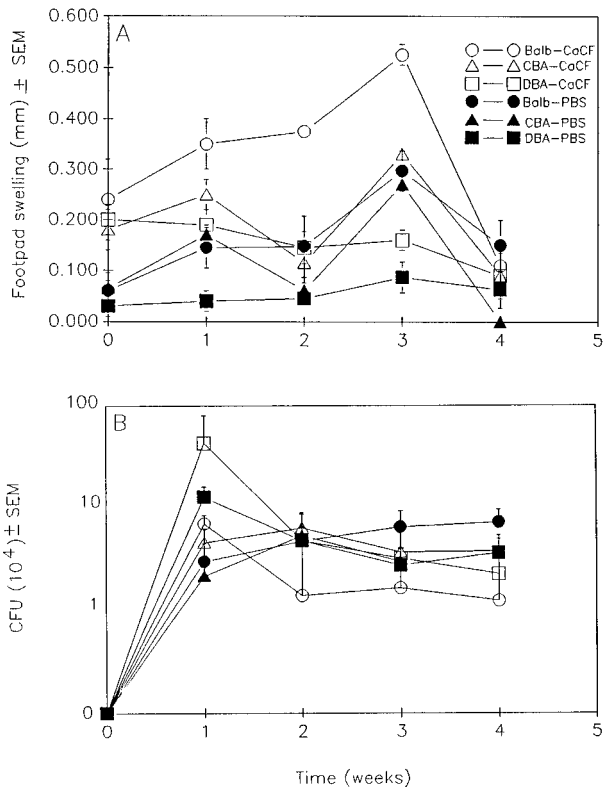

FIG. 1. Effects of preinduced *Candida*-specific DTH on experimental *C. albicans* vaginitis in strains of mice with different susceptibilities to *C. albicans* infection. Seven days after groups of mice were immunized with CaCF-CFA or PBS-CFA (control) (week 0), the mice were vaginally inoculated with *C. albicans*. Weekly estrogen treatments began 72 h prior to vaginal inoculation. CaCF-CFA-immunized and PBS-CFA-treated BALB/cJ, CBA/J, and DBA/2 mice were monitored for 4 weeks for DTH (A) and vaginal *C. albicans* burden (B). Separate groups of five mice were used at each time point. The mean footpad swelling (DTH) (A) and CFU (B) ± the standard errors of the mean (SEM) from three experiments are shown.

CFA (PBS-CFA). Four days after immunization, the mice were treated with the first of weekly subcutaneous injections of 0.5 mg of estradiol valerate (Sigma Chemical Co., St. Louis, Mo.) to induce and maintain pseudoestrus. Seven days after immunization, the mice were given a vaginal inoculation of *C. albicans* (5×10^5 blastoconidia per mouse), using strain 3153A (5). DTH reactions (footpad swelling following challenge with CaCF), vaginal fungal burden (quantitative vaginal lavage fluid culture), and wet-mount slide preparations of lavage fluid from separate mice were monitored weekly for 4 weeks. The qualitative content of hyphae on wet-mount slides was scored in a blinded manner by using a scale of 0 to 4+, where 4+ represented large sheets of hyphae and 0 referred to the absence of hyphae. The cumulative results of three experiments (five mice per group with separate groups of mice employed at each time period) are illustrated in Fig. 1. At the time of vaginal inoculation (week 0), BALB/cJ, CBA/J, and DBA/2 CaCF-CFA-immunized mice had increased DTH compared with the PBS-CFA-treated mice of the same strain (Fig. 1A) ($P < 0.05$, 0.03, and 0.0008, respectively). Within 1 week of vaginal inoculation, DTH in CaCF-CFA-immunized mice was still significantly higher than that of their PBS-CFA control counterparts ($P < 0.008$, 0.03, and 0.02, respectively) that had begun to show increased DTH as a result of the local vaginal infection (5). During weeks 2 and 3 post-vaginal inoculation, DTH in all PBS-CFA-treated mice had increased such that only BALB/cJ CaCF-CFA-immunized mice had higher reactivity compared with its PBS-CFA-treated control group ($P < 0.004$ and 0.01, respectively). During weeks 1 to 4, the levels of DTH among the three strains of *Candida*-immunized or PBS-CFA-treated mice were generally not significantly different from one another, although BALB/cJ mice usually had the highest levels of DTH, followed by CBA/J and DBA/2 mice. This differential trend in DTH among the three strains of mice together with the specific times at which statistically significant differences were observed (weeks 2 and 3 for CaCF-immunized mice and week 1 for PBS-CFA-treated mice) are consistent with the general pattern of resistance to systemic *C. albicans* infections for the three strains (BALB/cJ > CBA/J > DBA/2) (9).

Despite the observed differences in *Candida*-specific DTH among the three strains of mice prior to and during a primary vaginal infection and the documented patterns of resistance and susceptibility to systemic *C. albicans* infections (9), the vaginal *C. albicans* burden in each of the three strains of preimmunized (CaCF-CFA) mice was not different from that in their control counterparts (PBS-CFA treated) throughout the infection period. There were also no observed differences in fungal burden among the three strains of mice. This was indicated by quantitative culture (Fig. 1B) and hyphal scores of vaginal lavage fluid which were in the 3+ to 4+ range for all mice. Thus, contrary to what may have been expected with regard to potential levels of infectivity in the three strains of mice, these results suggest that vaginitis is similar in BALB/cJ, CBA/J, and DBA/2 mice and that preinduced *Candida*-specific systemic CMI in each murine strain is equally nonprotective against vaginal *C. albicans* infections.

Effects of murine strain on partial protection from a second *C. albicans* vaginal infection. BALB/cJ, CBA/J, and DBA/2 mice were first given a primary vaginal inoculation (5×10^5 *C. albicans* blastoconidia) in the absence of estrogen (2). At biweekly intervals beginning at week 2, groups of four to five mice from each strain were monitored for DTH and sacrificed, vaginal lavages were performed, and the lavage fluid was examined microscopically and cultured. Primary-infected nonestrogen-treated mice from all murine strains showed complete and spontaneous clearance of the vaginal infection by the fourth week, as indicated by sterile lavage fluid and no microscopic evidence of hyphae. During the third week following the primary infection (day 25), mice began receiving estrogen (0.5 mg per mouse). They were then given a second vaginal inoculation at week 4 (day 28) with 5×10^4 *C. albicans* blastoconidia (2). Positive control mice included animals given a primary vaginal inoculation of *C. albicans* at the time when the experimental mice were receiving the second inoculation. DTH, quantitative lavage cultures, and lavage fluid hyphal scores were monitored weekly for 2 weeks. In these experiments, estrogen treatment during the reinfection period was modified from a weekly (2) to a single administration, as recent data showed that the partial protection was better discriminated under slightly reduced estrogen conditions (unpublished observations). The cumulative results of three experiments (five mice per group with separate groups of mice employed at each time point) are illustrated in Fig. 2. During the primary infection in the absence of estrogen (first 4 weeks), levels of DTH for the three strains of mice were not statistically different (Fig. 2A), although responses at week 4 were similar to those observed with CaCF-CFA-immunized infected mice (BALB/cJ > CBA/J > DBA/2) (Fig. 1). During the week following the second *C. albicans* vaginal inoculation (week 5), anamnestic DTH was observed for all three strains of reinfected mice compared with primary-infected mice of the syn-

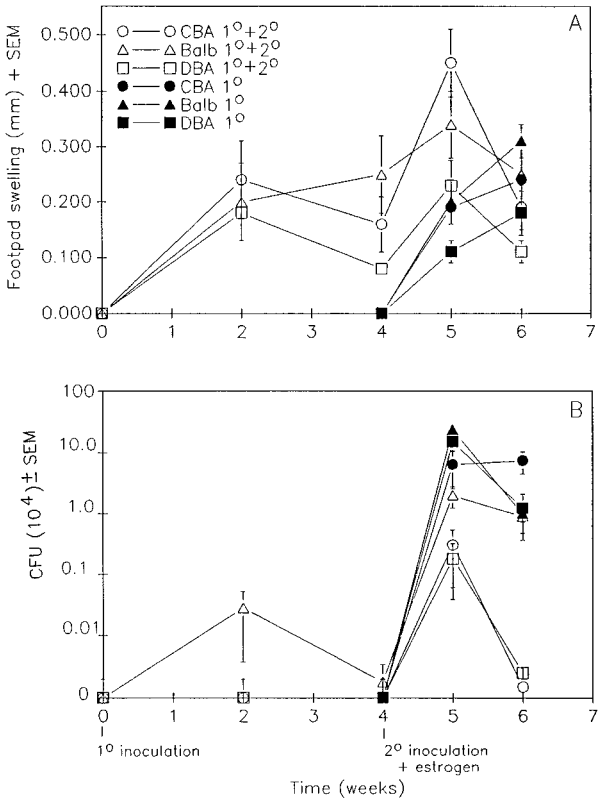

FIG. 2. Effects of murine strain on partial protection from reinfection. Groups of 10 BALB/cJ, CBA/J, and DBA/2 mice were given a primary vaginal inoculation of *C. albicans* in the absence of estrogen. DTH (A) and vaginal *C. albicans* burden (B) were monitored in separate groups of mice at weeks 2 and 4 (five mice per time point). At week 4, groups of 10 primary-infected mice of each strain were given a second vaginal inoculation in the presence of estrogen (given 72 h prior to inoculation). Control mice of each strain (10 mice per group) were given a primary vaginal inoculation also in the presence of estrogen at the time the experimental mice received the second inoculation. Footpad swelling representing DTH (A) and vaginal *C. albicans* burden (B) were monitored for 2 weeks (weeks 5 and 6) in separate groups of five mice each. Results for experimental mice (1° + 2°) were compared with results for control mice (1°) of the same strain. Shown are the mean results ± the standard errors of the mean (SEM) for three experiments.

geneic strain ($P < 0.02$, 0.001, and 0.02 for BALB/cJ, CBA/J, and DBA/2 mice, respectively), with no differences between the three strains of reinfected mice. At week 6 (2 weeks after the second inoculation), levels of DTH in reinfected mice were reduced but remained similar among the three strains of mice. Moreover, although the level of anamnestic DTH in reinfected mice was reduced during this second time point, the level of DTH in reinfected mice was indistinguishable from that in primary-infected control mice, consistent with our previous findings (2).

One week following the second inoculation (week 5), vaginal fungal burden within each strain was significantly lower in reinfected mice compared with their primary-infected control groups ($P < 0.0001$, 0.05, and 0.03 for BALB/cJ, CBA/J, and DBA/2 mice, respectively). Similar results were observed at week 6 for CBA/J and DBA/2 mice ($P < 0.001$ and 0.02, respectively), whereas BALB/cJ mice no longer showed significant differences in vaginal *C. albicans* burden. Comparisons among the three strains of mice at week 5 showed no significant differences in vaginal fungal burden among BALB/cJ, CBA/J, and DBA/2 mice with a primary vaginal infection or, similarly, among those mice which had been reinfected. Similar results were observed at week 6 among the three strains of primary infected mice but not for those reinfected, as vaginal fungal burden in reinfected BALB/cJ mice was significantly higher than that in CBA/J and DBA/2 mice. These results indicated that the partial protection against reinfection was achieved in DBA/2, CBA/J, and BALB/cJ mice, although the protective effect became limiting in BALB/cJ mice. Furthermore, during periods when partial protection was observed with all three murine strains, no significant differences were evident in the degree of protection among the strains. Thus, it does not appear that patterns of resistance and susceptibility to systemic *C. albicans* infections (9) similarly apply to protection against vaginal *C. albicans* infections. Interestingly, the reduction in anamnestic DTH levels in reinfected CBA/J and DBA/2 mice despite the continued evidence for local protection is similar in principle to the findings of previous studies in which partial protection against vaginitis was retained when systemic *Candida*-specific CMI was intentionally suppressed (2). This observation supports our contention that protection against vaginitis is locally rather than systemically derived.

Taken together, these results confirm and extend our previous findings regarding the lack of effects of systemic CMI against vaginal *C. albicans* infections in mice (2, 5). In preimmunized primary-infected mice, the levels of vaginal fungal burden and the lack of protection against vaginitis remained unchanged in mice more resistant or sensitive to systemic candidiasis. Similarly, the partial protection against a second vaginal infection in CBA/J mice (2) was observed equally with resistant and sensitive strains of mice. Thus, our results suggest that the differential patterns of susceptibility observed with BALB/cJ, CBA/J, and DBA/2 mice during systemic candidiasis (9) cannot be extended to vaginal *C. albicans* infections. Furthermore, the virtual lack of differences among the three strains of mice with respect to the effects of systemic CMI on *C. albicans* vaginitis provides additional support for the hypothesis that systemic CMI does not represent an important host defense mechanism at the vaginal mucosa. We hypothesize that local (compartmentalized) acquired host defense mechanisms (T lymphocytes together with neutrophils, macrophages, and related cytokines) are important for protection against vaginal *C. albicans* infections. If so, the lack of differences in protection among the three strains of mice suggests that the immune-related patterns of resistance and susceptibility to systemic *C. albicans* infections may not apply to mucosal immune mechanisms against vaginal *C. albicans* infections.

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