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Mice Immunized by Primary Vaginal *Candida albicans* Infection Develop Acquired Vaginal Mucosal Immunity

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It has been postulated that systemic cell-mediated immunity (CMI) is an important host defense mechanism against *Candida* infections of the vagina. However, in an estrogen-dependent murine model of experimental vaginal candidiasis, we recently showed that systemic *Candida*-specific Th1-type CMI induced by immunization with *Candida* culture filtrate antigen had no effect on vaginal *Candida* population levels during the course of a vaginal infection. In the present study, mice given a second vaginal inoculation in the presence of peripheral *Candida*-specific Th1-type CMI induced by prior vaginal infection had anamnestic-type increased delayed-type hypersensitivity (DTH) responses, concomitant with significantly fewer *Candida* organisms in the vagina than in primary-infected mice. In addition, organisms in secondary-infected mice were fragmented and superficial penetration into the epithelium was reduced. The systemic presence of *Candida*-specific T suppressor (Ts) cells that significantly suppressed the infection-derived anamnestic DTH reactivity did not abrogate the protective effect in the vagina. Additional experiments showed that vaginally immunized mice were not protected from gastrointestinal or systemic candidiasis and, in contrast to mice with a second vaginal infection, did not demonstrate anamnestic DTH reactivity. These results suggest that a moderate level of local protection against a *Candida* vaginal infection can be achieved by vaginal immunization but that the protective role of acquired peripheral *Candida*-specific Th1-type reactivity at the vaginal mucosa appears to be limited.

Recurrent vulvovaginal candidiasis (RVVC) is an opportunistic mucosal infection that affects up to 5% of otherwise healthy women of child-bearing age (16–18) and is most often caused by *Candida albicans* (11, 23, 27, 34). Short-term conventional topical or systemic antifungal therapy, although effective in eradicating individual attacks, does not prevent recurrence in women with chronic or recurrent vaginitis. Because the incidence of mucosal candidiasis is high in individuals with AIDS (20, 22) and lymphoma (14) and those treated with corticosteriods (21), and because patients with chronic mucocutaneous candidiasis often present with depressed cell-mediated immunity (CMI) (10, 28), it has been suggested that RVVC involves a breakdown of *Candida*-specific CMI. It is unclear, however, whether the putative breakdown of CMI is localized at the vaginal mucosa or is derived in the peripheral circulation. Evidence for the former includes the fact that individuals with chronic mucocutaneous candidiasis who are susceptible to oral and cutaneous candidiasis are not susceptible to vaginitis (27), whereas women with RVVC who are susceptible to intractable bouts of vaginitis are not susceptible to oral, esophageal, or cutaneous candidiasis (34). Several studies which examined systemic CMI reactivities (*Candida*-specific cutaneous skin test reactivity and peripheral blood lymphocyte proliferation and lymphokine production) in women with RVVC were controversial but showed in many cases that CMI expressed in the peripheral circulation of women with RVVC was normal (9, 12, 15, 35–37).

With an estrogen-dependent murine model of experimental vaginal candidiasis, considerable information has been obtained regarding the role of *Candida*-specific systemic CMI expressed in the periphery as a host defense mechanism for the vaginal mucosa. Results showed that mice given a vaginal inoculum of *C. albicans* under conditions of pseudoestrus acquire a persistent vaginal infection, while mice inoculated in the absence of estrogen resolve the infection within 3 weeks (6, 7). In addition, regardless of the estrogen status, vaginally inoculated mice express sustained *Candida*-specific Th1-type CMI in the periphery, detected in vivo by delayed-type hypersensitivity (DTH) responses and in vitro by lymph node cell-mediated interleukin-2 and gamma interferon production in response to *Candida* antigens (6, 7). However, further studies with this model showed that the presence of induced Th1-type *Candida*-specific CMI in the periphery had no effect on vaginal *Candida* burden through 4 weeks of infection (8). These results suggested that systemic CMI expressed in the periphery may be independent of mucosal CMI expressed in the vagina.

To further address the issue of whether peripheral Th1-type CMI is protective at the vaginal level, we asked whether *Candida*-specific Th1-type CMI expressed in the periphery as a result of a primary vaginal infection could modify the natural history of a second vaginal infection.

MATERIALS AND METHODS

Mice. Female CBA/J ($H\text{-}2^k$) mice, 8 to 10 weeks of age, purchased from the Jackson Laboratories (Bar Harbor, Maine), were used throughout these studies.

Antigens. *C. albicans* culture filtrate antigen(s) (CaCF) was prepared as previously described (6) by using *C. albicans* 3153A. Briefly, supernatants from a 3-day *C. albicans* culture grown in <12,000-molecular-weight phytone-peptone/glucose dialysate medium was concentrated 10-fold on a 10,000-molecular-weight-exclusion membrane (Amicon Corp., Danvers, Mass.) while washing with 1 to 2 volumes of phosphate-buffered saline (PBS). The final filtered preparation had a protein concentration of 0.2 to 0.5 mg/ml, as measured by the trichloroacetic acid precipitation kit (Sigma Chemical Co., St. Louis, Mo.). The *Limulus* amebocyte lysate test (Sigma) showed that CaCF had negligible (equal to or less than 0.06 U/ml) levels of endotoxin.

Vaginal immunization (primary infection) and DTH reactivity. Nonestrogenized mice were given a vaginal inoculation of viable stationary-phase *C.*

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albicans blastoconidia as previously described (6, 7). Briefly, 5×10^5 *C. albicans* blastoconidia were inoculated into the vaginas of mice in a volume of 20 μl of PBS. Control mice received 20 μl of PBS in the vagina. At weekly intervals beginning 1 week after inoculation, groups of four or five mice were footpad challenged with 10 μg (50 μl) of CaCF as previously described (6, 7) (one footpad received CaCF and the contralateral pad received PBS after measurement of the footpads with a micrometer). The footpad swelling was measured 18 to 24 h later, and the mice were killed thereafter.

Enumeration of *C. albicans* from the vaginas of infected mice. Vaginal *Candida* burden was quantitated as previously described (6, 7). Briefly, vaginal lavages were performed on dead mice with 100 μl of PBS, and the lavage fluid was serially diluted and plated onto Sabouraud dextrose (SAB) agar. The CFU from lavage cultures after 48 to 72 h were used to determine vaginal *Candida* burden. Vaginal lavage fluid was also analyzed microscopically (wet-mount slide preparation) for the presence of hyphae by a blinded observer. Hyphal scores were from 0 for no hyphae to ++++ for large hyphal masses.

Secondary vaginal infection. Four weeks following a primary vaginal inoculation in the absence of estrogen, mice were given a second vaginal *Candida* inoculation in the presence of pseudoestrus. For these studies, clearance of the primary *Candida* infection was first confirmed by negative vaginal lavage culture. Mice were then injected subcutaneously with 0.5 or, in specific experiments, 0.2 mg of estradiol valerate in 0.1 ml of sesame oil 72 h prior to the second vaginal inoculation (6, 7). Estrogen treatments were continued on a weekly basis until completion of the experiment. The inoculum for the second vaginal inoculation was identical to or 10-fold lower than that for the original inoculation (5×10^5 or 5×10^4 *C. albicans* blastoconidia in 20 μl of PBS). Two sets of controls were included. The negative control consisted of primary-infected, nonestrogenized mice that were subsequently estrogenized and received 20 μl of PBS in the vagina instead of the second *Candida* inoculum. The positive control consisted of estrogenized mice given a primary *C. albicans* vaginal inoculation (5×10^5 or 5×10^4 blastoconidia) at the time that other groups of mice received the second *C. albicans* inoculation. In any experiment, the primary-infected positive control mice received the same number of blastoconidia as the mice receiving the secondary inoculation. These primary-infected positive control mice had received only PBS in the vagina during the nonestrogenized infection period. Beginning 1 week after the first vaginal inoculation and continuing for 3 weeks, randomized mice were assessed for DTH reactivity and sacrificed, and vaginal lavages were performed as described above.

Histopathology. Vaginas from randomized, nonlavaged mice were excised, fixed in 10% Formalin, and embedded in paraffin. Paraffin sections were examined serially until high concentrations of hyphae were located. Subsequent sections were analyzed by silver staining methods with a commercial kit (Sigma).

Suppressor cell induction. To induce *Candida*-specific tolerance by T suppressor (Ts) cells, mice were injected intravenously (i.v.) with 100 to 200 μg of CaCF as previously described (8). Mice were injected with CaCF at one of two times during the primary infection: either 1 week prior to the primary vaginal inoculation (Ts-wk1) or 1 week prior to the second vaginal inoculation (Ts-wk3). Different tolerance induction times were used to achieve functional *Candida*-specific Ts cells at the time of either the primary or secondary vaginal inoculation. Ts cell activity was monitored weekly by DTH reactivity, beginning at the time of primary or secondary vaginal inoculation.

GI infection. In specific experiments, naive or vaginally immunized mice with positive DTH reactivity were given a gastrointestinal (GI) infection. For these experiments, following clearance of a primary vaginal infection in the absence of estrogen, groups of four to five mice were inoculated with 2×10^7 viable *C. albicans* blastoconidia in 0.1 ml of PBS with a 22-gauge, 1.5-in. (ca. 4-cm) feeding needle (Popper and Sons, Inc., New Hyde Park, N.Y.) (3). The positive control consisted of nonestrogenized mice that received PBS in the vagina followed by a *Candida* inoculation into the GI tract. DTH reactivity, measured weekly for 2 weeks, was used as an indicator of in vivo CMI reactivity. Weekly stool specimens were collected aseptically and placed in sterile PBS. The stool contents were homogenized in a Stomacher lab blender (model 80; Tekmar Corp., Cincinnati, Ohio), serially diluted, and plated onto SAB agar. The number of *C. albicans* CFU after 48 h of incubation was used as a measure of *C. albicans* in the GI tract (26). Results were expressed as CFU per gram of stool. It should be noted that GI-infected mice were tested longitudinally during the 2-week period, with alternate footpads used for the weekly challenge and detection of DTH reactivity.

Systemic infection. In specific experiments, naive or vaginally immunized mice were given a systemic inoculation of viable *C. albicans* blastoconidia. For these experiments, groups of five mice were inoculated i.v. with 2×10^5 *C. albicans* blastoconidia in 0.2 ml of PBS (31) following clearance of the primary vaginal infection. The positive control consisted of nonestrogenized PBS-treated (vaginally) mice given a similar i.v. inoculation. DTH reactivity was measured 1 week following systemic inoculation in both groups of mice. Following footpad measurements, the mice were sacrificed, and the kidneys, spleen, and liver were collected from each mouse and placed in sterile PBS. The organs were homogenized in a Stomacher blender, serially diluted, and plated onto SAB agar. Colonies were enumerated after 48 h of incubation. Results are expressed as CFU of *C. albicans* per organ.

Statistical analysis. Median DTH reactivity or vaginal *Candida* burden for individual groups of mice was analyzed by the Mann-Whitney U test. Differences in mean DTH reactivity or vaginal *Candida* burden were analyzed by Student's *t* test. Significant differences were defined as $P < 0.05$.

RESULTS

Effect of vaginal immunization on a second vaginal infection. Using an estrogen-dependent murine model of vaginal candidiasis, we have previously demonstrated that mice given a vaginal inoculation of *C. albicans* in the absence of estrogen acquire an infection that resolves within 3 weeks but develop and sustain Th1-type peripheral CMI similar to that observed in estrogenized infected mice (7). In the present study, we asked whether nonestrogenized mice that had cleared a primary *C. albicans* vaginal infection were completely or partially protected from a second vaginal infection under the condition of pseudoestrus. The rationale for the use of estrogen in these experiments was to monitor the second infection in an environment suitable for growth and subsequent detection of *Candida* organisms in the vagina. Results were compared with those for control estrogenized mice given a primary vaginal infection. DTH reactivity and vaginal *Candida* burden were monitored for 4 weeks during the primary infection and for 3 weeks following the second vaginal inoculation.

In accord with our previous studies (6, 7), the primary infection was characterized by peak DTH reactivity (0.35 ± 0.06 mm) during the second week and remained positive during the third (0.29 ± 0.05 mm) and fourth (time of reinoculation; 0.22 ± 0.03 mm) weeks. The vaginal *Candida* burden was low ($2.0 \times 10^3 \pm 1.7 \times 10^3$ CFU/100 μl of lavage fluid) after the first week and declined to undetectable levels by the third week.

For secondary vaginal infection studies, experimental mice received two inoculations of *C. albicans*, one under nonestrogenized conditions and the second under estrogenized conditions at week 4. Positive control mice received PBS in the vagina under nonestrogenized conditions, followed by a primary vaginal infection under estrogenized conditions at week 4. Negative control mice received a primary vaginal infection under nonestrogenized conditions and PBS in the vagina under estrogenized conditions at week 4. The results in Fig. 1 illustrate the DTH reactivity and vaginal *Candida* burden in infected mice during the 3 weeks following the second vaginal treatment in the presence of estrogen. One week after experimental mice received the second vaginal *Candida* inoculation, the median DTH reactivity was significantly higher than that observed in positive control mice with a primary vaginal infection (Fig. 1A; $P < 0.0025$). During weeks 2 and 3, the DTH reactivity in mice with a second vaginal infection remained at levels similar to those in week 1, while DTH reactivity in primary-infected mice increased to levels equivalent to those in mice with a second vaginal infection. In contrast, negative control mice originally infected without estrogen for 4 weeks and then given PBS at the time that other mice received the second *Candida* inoculation had low to negligible DTH reactivity throughout the 3-week period (reflective of weeks 5, 6, and 7 following vaginal inoculation).

Vaginal *Candida* burden in experimental mice was significantly lower than that in positive control mice during the 3-week period (Fig. 1B; $P < 0.0057$, 0.041, and 0.03 for weeks 1, 2, and 3, respectively). These results were supported by vaginal lavage fluid hyphal scores, which ranged from 0 to +++ for secondary-infected mice and +++ to ++++ for control primary-infected mice. Negative control mice originally infected in the absence of estrogen and then estrogenized and given PBS in the vagina had no evidence of additional vaginitis, either by vaginal lavage cultures or by microscopic examination of vaginal lavage fluid. The histopathology results for randomly

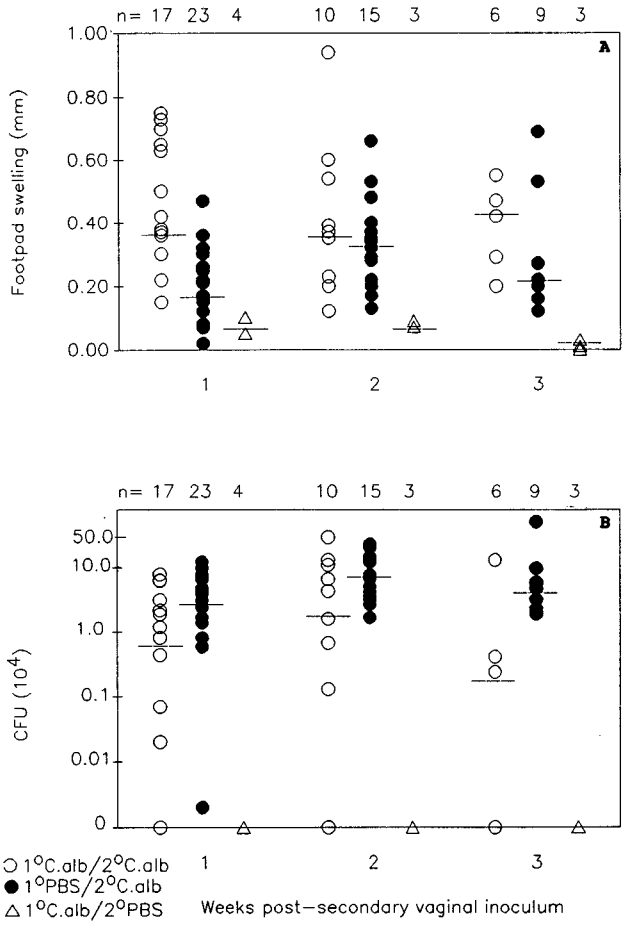

FIG. 1. Protection from vaginal candidiasis by primary vaginal immunization in nonestrogenized mice. Four weeks after mice were given a primary (1°) vaginal inoculation of *C. albicans* (5×10^5 stationary-phase blastoconidia) and the vaginal infection had cleared, the mice were reinoculated (5×10^5 stationary-phase blastoconidia) in the presence of estrogen. Control mice either received a primary vaginal inoculation in the presence of estrogen or were given PBS in the vagina instead of the secondary (2°) vaginal inoculation. DTH reactivity and vaginal *Candida* burden were monitored for 3 weeks. (A) Footpad swelling responses, representing DTH reactivity. (B) CFU of *C. albicans* from vaginal lavage cultures. The figure shows the results for up to four experiments.

selected nonlavaged vaginas were supportive of those from quantitative cultures. The results illustrated in Fig. 2 show that hyphae present in mice 1 week after secondary inoculation were appreciably more fragmented, with reduced superficial penetration into the cornified epithelium (Fig. 2B) than was observed in primary-infected mice (Fig. 2A). Proportional results were observed during the second week (Fig. 2C and D), although hyphal fragmentation was increased in both primary- and secondary-infected mice.

To determine whether the differences in vaginal *Candida* burden would be more pronounced with a smaller inoculum or a lower dose of exogenous estrogen, mice that were to receive a secondary or control primary inoculation were given 5×10^4 *Candida* blastoconidia (10-fold fewer) in the presence of 0.2 mg of estradiol valerate (2.5-fold less). The results showed that for two experiments (six mice per group), the differences in vaginal lavage culture CFU between secondary-infected and control primary-infected mice were indeed more discrete (mean CFU: week 1, $2.3 \times 10^4 \pm 5.8 \times 10^3$ versus $1.5 \times 10^5 \pm 6 \times 10^4$, $P < 0.04$; week 2, $2.4 \times 10^4 \pm 1 \times 10^4$ versus $8.0 \times 10^4 \pm 3 \times 10^4$, $P < 0.05$, respectively). These results were confirmed by vaginal lavage fluid hyphal scores. In these experiments, the pattern of DTH reactivity for secondary-infected and control primary-infected mice at 1 week post-vaginal inoculation was similar to that in experiments performed with the larger vaginal inoculum and higher dose of exogenous estrogen (0.47 ± 0.09 mm versus 0.17 ± 0.05 mm, $P < 0.0008$). Vaginal protection could not be evaluated in the absence of exogenous estrogen because of extremely low levels of vaginal *Candida* burden in both secondary-infected and control primary-infected mice during the 2-week period. However, DTH reactivities in these nonestrogenized mice were similar to those in mice reinfected in the presence of exogenous estrogen.

Effect of suppressed *Candida*-specific systemic CMI on protection from a second vaginal infection. To determine whether *Candida*-specific CMI expressed in the periphery was responsible for the lower vaginal *Candida* titers in mice with a second vaginal infection, secondary vaginal infections were monitored for mice whose *Candida*-specific peripheral CMI was suppressed. To achieve suppression of *Candida*-specific CMI, mice were treated with CaCF to induce *Candida*-specific Ts cells (8) either 1 week prior to primary vaginal inoculation (Ts-wk1) or 1 week prior to secondary vaginal inoculation (Ts-wk3). The median DTH reactivities and vaginal *Candida* burden for 2 weeks following secondary vaginal inoculation are shown in Fig. 3. In comparison to untreated mice, mice treated with CaCF prior to the primary or secondary vaginal inoculation had significantly suppressed DTH reactivity throughout the 2-week period (Fig. 3A; $P < 0.0031$ and 0.02, respectively). However, vaginal *Candida* burden was similar in secondary-infected mice irrespective of the level of *Candida*-specific DTH reactivity (Fig. 3B). Vaginal lavage fluid hyphal scores for each group of animals ranged from 0 to +++.

Effect of vaginal immunization on GI and systemic candidiasis. To determine whether the *Candida*-specific peripheral CMI generated from a vaginal infection could modify *Candida* population levels in mice with either GI or systemic candidiasis, nonestrogenized mice were inoculated with *C. albicans* either orally by a feeding tube or i.v. 4 weeks after a primary vaginal inoculation. DTH reactivity and *C. albicans* titers in stool samples (GI) or organs (systemic) were determined for 1 to 2 weeks. The results for *Candida* burden in stool and kidney samples are shown in Table 1. The results show that the number of *C. albicans* CFU in the GI tract of mice given a primary GI inoculation was not different from that in the GI-inoculated mice that were previously exposed to *C. albicans* by vaginal infection. The results for weeks 2 and 3 were not appreciably different from those at week 1 (data not shown). Similar results were observed for animals with systemic infections. *C. albicans* CFU in the kidneys of mice given a primary systemic infection were not different from those in systemically inoculated mice previously exposed to *C. albicans* in the vagina. The results were similar for CFU of *C. albicans* in the livers and spleens as well, although the *Candida* burden was considerably less (data not shown).

DTH reactivity measured in these animals is illustrated in Table 2. DTH reactivity observed in GI-infected mice previously exposed to *C. albicans* in the vagina was similar to that in GI-inoculated positive control mice. In the case of systemic infection, DTH reactivity was negligible in all animals. It should be noted that all systemically infected mice were lethargic and had lost considerable weight at the time of DTH testing. Animals randomized for evaluation at the 2- and 3-week intervals did not survive past 10 days.

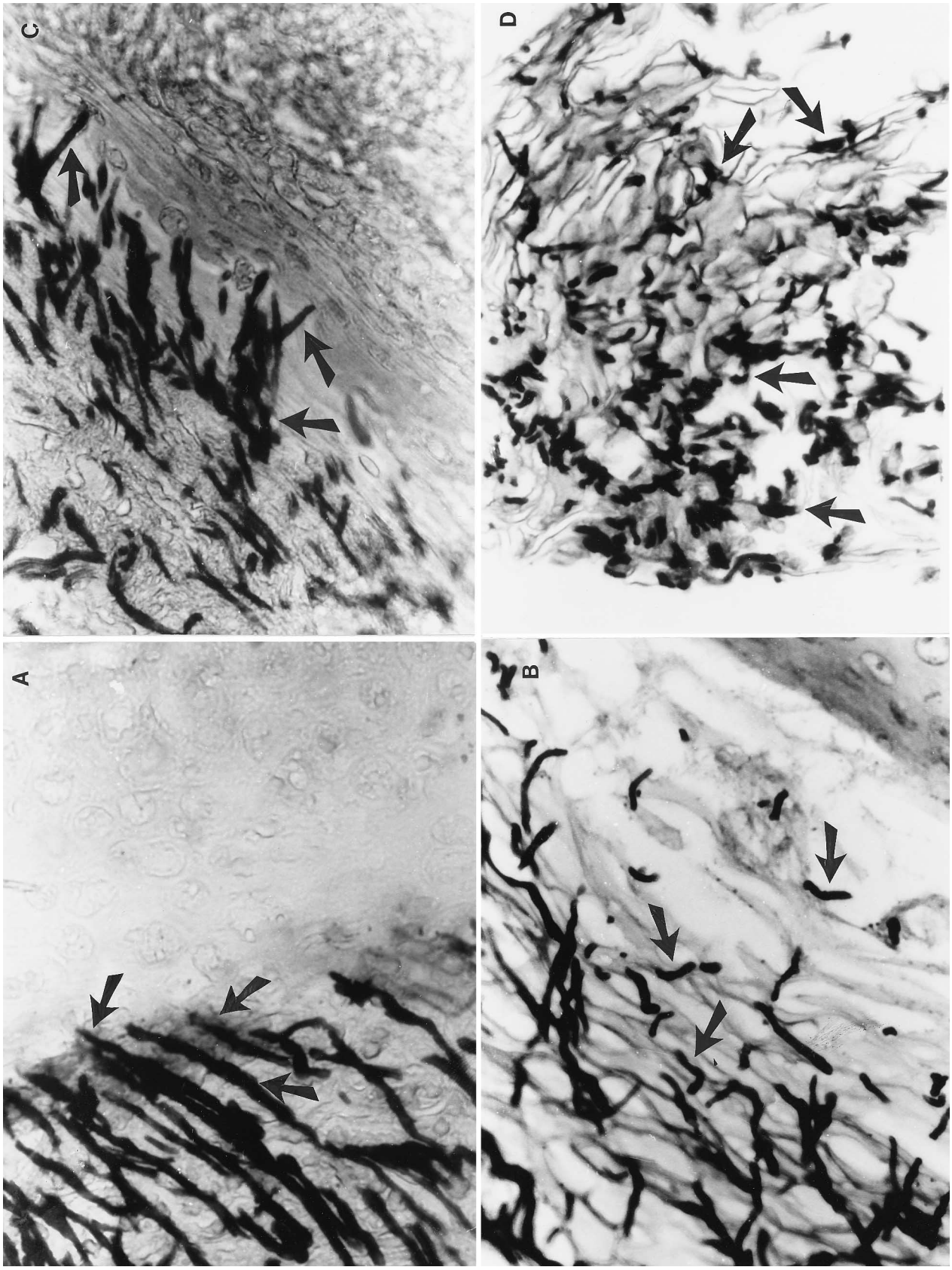

FIG. 2. Vaginas from randomly selected, nonlavaged, primary- and secondary-infected mice were excised, fixed in Formalin, and embedded in paraffin. Paraffin sections were cut onto slides and analyzed by silver stain. Representative sections of vaginal lumens from primary (A and C)- and secondary (B and D)-infected mice 1 (A and B) or 2 (C and D) weeks after the respective vaginal inoculations are shown. Arrows point to representative hyphae.

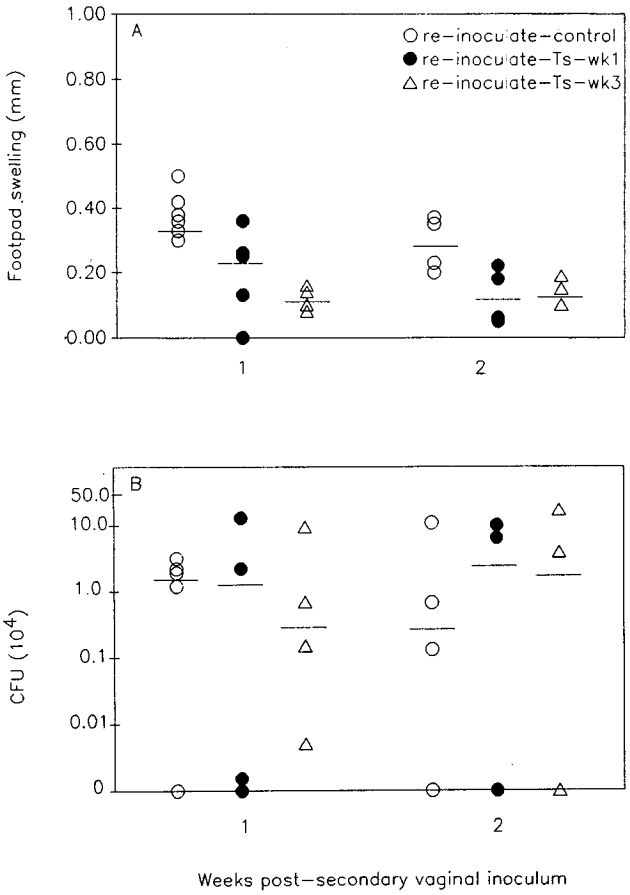

FIG. 3. Effects of *Candida*-specific Ts cells on protection from secondary vaginal *Candida* infection. Mice were treated with CaCF to induce *Candida*-specific Ts cells either 1 week prior to primary vaginal inoculation (Ts-wk1) or 3 weeks after primary vaginal inoculation (Ts-wk3) in nonestrogenized mice. Primary vaginal inoculation consisted of 5×10^5 stationary-phase blastoconidia. At the fourth week, mice were reinoculated with *C. albicans* (5×10^5 stationary-phase blastoconidia) in the presence of estrogen. DTH reactivity and vaginal *Candida* burden were monitored for 2 weeks. (A) Footpad swelling responses, representing DTH reactivity. (B) CFU of *C. albicans* from vaginal lavage cultures. The figure shows the results for up to two experiments (Ts-wk3 was only included in one experiment as a confirmation of our original observations).

DISCUSSION

Results from studies of women with RVVC and experimental animal models show that *Candida* vaginitis can occur in the presence of detectable levels of *Candida*-specific Th1-type CMI expressed in the periphery (6–9). These results have led us to postulate that local rather than systemic host defense mechanisms provide protection at the vaginal mucosa. Since many normal healthy women are chronically colonized with *C. albicans* in the vagina but do not acquire symptomatic vaginitis (34), such local protective host defense mechanisms most certainly exist. It remains unknown, however, what mechanisms constitute vaginal protection and whether *Candida*-specific immunity (local or systemic) generated by vaginal immunization can provide significant protection against *Candida* vaginitis. To address this question, we investigated whether mice which spontaneously resolve a primary vaginal infection and express *Candida*-specific Th1-type peripheral CMI could be partially or completely protected from a second vaginal infection.

Results showed that in comparison to estrogenized mice with a primary vaginal infection (positive control), mice given a second vaginal infection had enhanced DTH reactivity concomitant with significantly lower *Candida* titers in the vagina. The patterns of DTH responses in nonestrogenized primary-infected mice (negative control), which peaked at 2 weeks, remained positive at week 4 in the absence of a detectable vaginal *Candida* burden, and became negligible in weeks 5 to 7, were similar to our previous findings for estrogenized mice (6). Furthermore, DTH responses elicited by CaCF in these mice were characterized by early and late swelling reactivities (6) normally observed in DTH. However, whereas estrogenized mice are characterized by low levels of DTH reactivity in the continued presence of *C. albicans* (weeks 6 to 10 post-vaginal inoculation) (6), the lack of DTH reactivity in nonestrogenized mice as early as week 5 presumably reflects the lack of additional antigen to support continued expression of DTH. On the other hand, the anamnestic pattern of DTH reactivity in mice with a second vaginal infection is synonymous with or typical of a secondary immune response, characterized by swelling reactivity that occurred early (1 week) and was sustained throughout the observed infection period. This type of reactivity suggests that putative memory T cells were present in mice receiving a second vaginal inoculation. This is supported by the low but nevertheless positive DTH reactivity at the time of the second vaginal inoculation (week 4).

The results of quantitative cultures in primary- and secondary-infected mice were supported by histopathology of vaginal tissue, in which secondary-infected mice had hyphae which were progressively more fragmented with reduced superficial penetration into the epithelium compared with hyphae in primary-infected mice. Taken together, these results indicate that vaginal immunization can modify the natural history of a *Candida* vaginal infection and that partial protection against vaginal candidiasis can be achieved. We recognize, however, that the bias of the model toward infectivity (i.e., permissive influence of estrogen and artificial inoculation of large numbers of organisms into the vagina) may influence and mask a more pronounced protective effect.

The lower vaginal *Candida* titers, together with anamnestic DTH reactivity in mice exposed to *Candida* organisms in the vagina a second time, initially suggested that there may have been an association between the protection and the peripheral Th1-type CMI induced by vaginal immunization. Indeed, *Candida*-specific Th1-type CMI expressed in the periphery has been shown to protect mice against lethal systemic candidiasis (31, 32). Moreover, in experimental murine models of listeriosis (4, 5) and leishmaniasis (24), protection from and resistance to secondary infections have been shown to occur concomitant with anamnestic CMI responses. However, in this study, the presence of *Candida*-specific Ts cells that suppressed the DTH reactivity in secondary-infected mice did not abrogate the partial protection from vaginitis. It should be noted that although we believe and have data to support the concept that i.v. injection of CaCF induces Ts cells (8), we recognize that suppression may alternatively or in part occur by additional mechanisms such as anergy, reticuloendothelial system blockade, negative regulatory cytokines, or other types of regulatory cells. Regardless of the mechanism(s), and although we cannot exclude the unlikely possibility that the greater than 60% suppression of DTH reactivity was not sufficient to abrogate the partial vaginal protection, these results represent yet another example whereby *Candida*-specific peripheral CMI, as detected by DTH reactivity, does not appear to play a significant role in events taking place at the vaginal mucosa. Alternatively, since DTH reactivity may not be entirely synonomous with protective immunity (13), it remains possible that the vaginal protection was derived from systemic CMI and that the

TABLE 1. Effect of vaginal immunization on GI and systemic candidiasis

Mouse no.	CFU in stool or kidney			
	GI infection[a]		Systemic infection[b]	
	Vaginal (first), GI (second)	GI (first)	Vaginal (first), systemic (second)	Systemic (first)
1	1.6×10^4	2.1×10^5	1.3×10^6	2.5×10^4
2	1.7×10^4	2.0×10^4	2.5×10^7	1.3×10^6
3	4.0×10^4	1.3×10^4	2.1×10^6	1.3×10^7
4	1.2×10^5	2.0×10^3	1.4×10^7	1.3×10^6
5	0	1.5×10^4	1.3×10^6	3.8×10^5
6	1.6×10^5	5.2×10^4		
7	2.7×10^5	7.0×10^5		
8		3.5×10^6		
Mean ± SEM	$8.9 \times 10^4 \pm 4.9 \times 10^4$	$5.6 \times 10^5 \pm 6.0 \times 10^5$	$8.7 \times 10^6 \pm 5.2 \times 10^6$	$3.2 \times 10^6 \pm 2.7 \times 10^6$

[a] Inoculum, 2×10^7 blastoconidia inoculated by feeding tube. Results are expressed as CFU of *C. albicans* per gram of stool sample taken 1 week following inoculation. The results are from two experiments.

[b] Inoculum, 2×10^5 blastoconidia injected i.v. The results show *Candida* burden in kidney, expressed as CFU of *C. albicans* per organ 1 week following inoculation. The results are from one experiment.

protective immunity was unaffected by the Ts cells. However, recent data from our laboratory (submitted for publication) showed that in vivo depletion of systemic CD4 and/or CD8 cells also had no effects on primary or secondary *Candida* vaginitis. Thus, while these results do not refute the concept of non-DTH-associated protective immunity, they suggest that the vaginal protection was not derived from systemic CMI.

The lack of observed vaginal protection by *Candida*-specific peripheral CMI could be explained by (i) the presence of nonimmunoprotective antigens which induced nonimmunoprotective Th1-type reactivity in the periphery or (ii) the presence of true immunoprotective CMI that could not traffic effectively into the vaginal mucosa. The former is unlikely, since the *Candida*-specific Th1-type reactivity induced by vaginal immunization was derived from viable organisms, which theoretically should have a strong tendency towards inducing immunoprotective CMI. However, whether one or both of these possibilities occur during a secondary vaginal infection, the data suggest that peripheral CMI did not provide vaginal protection. Alternatively, the protection may be entirely derived from the local environment of the vaginal mucosa. Indirect evidence to support this comes from the lack of anamnestic DTH reactivity concomitant with the lack of any observed protection in vaginally immunized mice challenged with GI or systemic *Candida* inoculation. In fact, mice given a systemic infection with or without vaginal immunization had little to no DTH reactivity, consistent with observations made by Rogers and Balish (30). The lack of protection against GI or systemic candidiasis in animals with existing peripheral *Candida*-specific Th1-type reactivity seemingly contradicts the evidence in animal models that supports a role for *Candida*-specific Th1-type $CD4^+$ T cells in acquired resistance to systemic (31–33) or GI (1, 2, 26) candidiasis. However, although preliminary in nature, the lack of anamnestic DTH reactivity following a second *Candida* exposure at a site different from the original one (vaginal followed by GI or systemic exposure) may indicate that for activation of protective secondary responses, exposure at the original site of inoculation is required. If so, the anamnestic DTH responses observed in the present study following two independent *Candida* exposures in the vagina may indicate the presence of memory cells in the vaginal mucosa that, upon activation, initiate protective host defenses for the vagina.

Locally acquired protective host defenses in the vaginal mucosa may consist of CMI and/or humoral immune mechanisms. Although the protective effect of humoral immunity is questioned, since the incidence of mucosal candidiasis is low in patients with B-cell disorders, Polonelli et al. recently showed that rats could be protected against *Candida* vaginitis by anti-idiotypic antibodies induced by intravaginal immunization with anti-*Candida* idiotypic antibodies (29). Thus, while humoral immunity cannot be excluded as a possible mechanism for the infection-derived protective effects, there are several factors that favor mechanisms that are CMI based. For example, the strong primary or secondary *Candida*-specific CMI reactivity detected in the periphery following local vaginal exposure to *Candida* organisms might equally be expressed in the local environment of the vagina. In support of this, α/β and γ/δ T cells have been identified in the vaginal epithelium of mice and are reportedly present in different percentages compared with those in the periphery (25). Additionally, in a guinea pig model of *Chlamydia trachomatis* genital infections, resistance to a second infection has been observed comcomitant with in vitro CMI reactivity by vaginal lymphocytes in response to chlamydial antigens (19). Studies are currently in progress to investi-

TABLE 2. Effect of vaginal immunization on DTH reactivity in mice with GI or systemic candidiasis

Mouse no.	DTH reactivity (mm)			
	GI infection[a]		Systemic infection[b]	
	Vaginal (first), GI (second)	GI (first)	Vaginal (first), systemic (second)	Systemic (first)
1	0.29	0.07	0.14	0.19
2	0.36	0.53	0.03	0.12
3	0.40	0.57	0.08	Died
4	0.37	0.67	0.01	0.05
5	0.46	0.63	0.03	0.01
6	0.05	0.32		
7	0.40	0.23		
8		0.21		
Mean ± SEM	0.33 ± 0.07	0.40 ± 0.11	0.058 ± 0.02	0.09 ± 0.04

[a] Inoculum, 2×10^7 blastoconidia inoculated by feeding tube. Results are expressed as footpad swelling responses 24 h after challenge with 10 μg of CaCF. Footpad challenge was performed 1 week following inoculation. The results are from two experiments.

[b] Inoculum, 2×10^5 blastoconidia injected i.v. Results are expressed as footpad swelling responses 24 h after challenge. Footpad challenge was performed 1 week following inoculation. The results are from one experiment.

gate these important local mechanisms which are protective against *Candida* vaginitis.

In summary, this report represents the first study showing that a vaginal *Candida* infection can immunize the vaginal mucosa and provide partial protection against a second episode of vaginitis. These data suggest that vaginal immunization can induce some form of locally acquired mucosal immunity and support the working hypothesis that local rather than peripheral host defense mechanisms are protective against vaginal candidal infections. In the light of information known about cell populations present in the vaginal mucosa, it is interesting to envision a concept of tissue-specific or compartmentalized host defense mechanisms that provide protection against *C. albicans* vaginal infections.

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