Effects of Preinduced Candida-Specific Systemic Cell-Mediated Immunity on Experimental Vaginal Candidiasis

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It has been postulated that systemic cell-mediated immunity (CMI) is an important host defense factor against recurrent vaginal infections caused by Candida albicans. Using an estrogen-dependent murine model of vaginal candidiasis, we have previously shown that mice inoculated vaginally with C. albicans acquire a persistent vaginal infection and develop Candida-specific Th1-type systemic CMI. In the present study, experimental vaginitis was monitored in the presence of preinduced systemic Candida-specific CMI. Mice immunized systemically with C. albicans culture filtrate antigens (CaCF) in complete Freund's adjuvant (CFA) had Th1-type reactivity similar to that of vaginally infected mice. CaCF given to mice intravenously induced Candida-specific suppressor T (Ts) cells. Mice preimmunized with CaCF-CFA and given a vaginal inoculum of C. albicans had positive delayed-type hypersensitivity (DTH) reactivity from the time of vaginal inoculation through 4 weeks. Conversely, mice infected in the presence of Ts cells had significantly reduced DTH responses throughout the 4-week period in comparison with naive infected mice. However, the presence of Th1-type Candida-specific DTH cells or Ts cells, either induced in mice prior to vaginal infection or adoptively transferred at the time of inoculation, had no effect on the vaginal Candida burden through 4 weeks of infection. A similar lack of effects was obtained in animals with lower Candida population levels resulting from a reduction in or absence of exogenous estrogen. These results suggest that systemic Th1-type CMI demonstrable with CaCF is unrelated to protective events at the level of the vaginal mucosa.

Recurrent vulvovaginal candidiasis (RVVC) is a mucosal infection frequently caused by Candida species that affects up to 5% of otherwise healthy women of childbearing age (22-24, 39). Candida albicans is the causative agent in approximately 85 to 90% of symptomatic, culture-positive RVVC patients (15, 22, 28, 32). Antimycotic agents, although effective in eradicating individual attacks, do not prevent recurrence in women with chronic or recurrent vaginitis. In contrast to infrequent episodes of vaginitis, which may be precipitated by pregnancy, oral contraceptives, uncontrolled diabetes mellitus, and particularly by the use of antibiotics, there are no recognized exogenous predisposing factors for RVVC (38). RVVC is presumed to result from diminished host defense mechanisms that increase susceptibility to symptomatic infection. Specifically, it is postulated that cell-mediated immunity (CMI) is a predominant host defense factor for mucosal surfaces and that the conversion of C. albicans from vaginal commensal to pathogen results from abnormalities in CMI. It is unclear, however, whether these abnormalities originate at the local mucosal surface of the vagina or at the systemic level, similar to those associated with chronic mucocutaneous candidiasis (1, 14, 33).

Clinical studies evaluating systemic CMI in patients with RVVC have produced controversial results (16, 20, 41-43). While there is general agreement that most symptomatic, culture-positive RVVC patients have reduced delayed cutaneous skin test reactivity to Candida antigens (16, 41), studies of in vitro proliferation of peripheral blood mononuclear cells have shown either reduced (20, 42, 43) or normal (16, 41) responsiveness to Candida antigens. In a comprehensive longitudinal study examining a large number of RVVC patients, we recently reported that peripheral blood lymphocyte CMI reactivity to multiple Candida antigens was not different from control responses, both during episodes of RVVC and during periods of infection-free remission (12). In addition, most of the women with reduced skin test reactivity to Candida antigens during episodes of RVVC reacquired normal reactivity shortly after successful treatment of vaginitis. Thus it appears that women with RVVC have normal peripheral CMI reactivity and that the transient reduction of skin test reactivity during episodes of RVVC is likely to be the result of infection rather than a factor predisposing them to recurrent vaginitis.

Immunological factors that predispose women to Candida vaginitis can be elucidated by use of animal models. Employing an estrogen-dependent murine model of experimental vaginal candidiasis, we recently began to characterize the CMI mechanisms associated with Candida vaginal infections. To this end, we have demonstrated that animals given a vaginal inoculum of C. albicans acquire a persistent vaginal infection and develop Candida-specific delayed-type hypersensitivity (DTH) reactivity which is indistinguishable from that elicited by CD4+ DTH T (T_{DTH}) cells induced by systemic immunization with Candida antigens (10). Studies to characterize this vaginal infection-induced systemic CMI reactivity revealed that lymph node cells from infected mice responded to Candida antigens with Th1-type reactivity (3, 13, 29), evident by the production of interleukin-2 (IL-2) and gamma interferon (IFN-γ), together with little to no IL-4 and IL-10 production (11). The objective of this study was to examine experimental vaginal candidiasis in the presence of preinduced Candida-specific systemic CMI.

MATERIALS AND METHODS

Mice. Female CBA/J (H-2b) mice, 8 to 10 weeks old, purchased from Jackson Laboratories, Bar Harbor, Maine, were used throughout these studies.

Antigens. C. albicans culture filtrate antigen(s) (CaCF) was
prepared as previously described (10) with *C. albicans* 3153A. Briefly, supernatants from a 3-day blastoconidium culture in a low-molecular-mass (<12,000- to 14,000-Da) dialysate medium were concentrated 10-fold by using a 10,000-molecular-weight exclusion membrane (Amicon Corp., Danvers, Mass.). Heat-killed *C. albicans* blastoconidia (HKB) were prepared by incubating a stationary-phase culture of *C. albicans* 3153A for 2 h at 60°C and then washing it twice with phosphate-buffered saline (PBS). *C. albicans* soluble cytoplasmic substances (SCS) (8) was a kind gift of Judith Domer, Tulane University School of Medicine, New Orleans, La. Cryptococcus neoformans culture filtrate antigen (CneF) was a kind gift of Juneann Murphy, University of Oklahoma Health Sciences Center, Oklahoma City, Okla. Purified protein derivative (PPD) of *Mycobacterium tuberculosis* was purchased from Parke-Davis Inc., Morris Plains, N.J. Dinitrofluorobenzene (DNFB) was purchased from Sigma Corp., St. Louis, Mo.

**Demonstration of Th1-type reactivity in mice immunized with CaCF.** Mice were immunized with 50 μg of a 1:1 emulsion of CaCF and complete Freund's adjuvant (CaCF-CFA) in a volume of 0.1 ml at each of two sites at the base of the tail as previously described (10). Seven days after immunization, spleen cells were removed and pooled from groups of three mice. Single cell suspensions (4 × 10⁶ cells per ml) were cultured with HKB (5 × 10⁷/ml) or SCS (125 μg/ml) as previously described (11). Culture supernatants were collected after 48 h of culture and stored at −70°C. IL-2 in the culture supernatants was quantitated by a bioassay using the IL-2-dependent CTLL (19) cell line (American Type Culture Collection, Rockville, Md.). Concentrations of IL-2 produced in response to *Candida* antigens were determined as previously described (11) by using known concentrations of recombinant human IL-2 (Cetus Corp., Emeryville, Calif.). IFN-γ in the culture supernatants was quantitated by a sensitive and specific enzyme-linked immunosorbent assay (Genzyme Corp., Cambridge, Mass.) as previously described (11). IL-4 in the culture supernatants was quantitated by a bioassay using the IL-4-dependent cell line CT.4S (21), kindly provided by William Paul, National Institutes of Health, Bethesda, Md. Concentrations of IL-4 produced by lymphocytes in response to *Candida* antigens were determined as previously described (11) by using known concentrations of recombinant murine IL-4 kindly provided by Immunex Corp., Seattle, Wash.

**Demonstration and characterization of suppression in mice made tolerant (tolerized) with CaCF.** Mice were injected intravenously (i.v.) with 100 to 200 μg of CaCF or culture filtrate medium as a control. Seven days later, mice were immunized with CaCF-CFA as described above. Six days after immunization, mice were footpad challenged with CaCF as previously described (10) and DTH reactivity was measured 24 h later. Suppression of DTH reactivity was calculated by the following equation: % suppression = [(positive control − test)/(positive control − negative control)] × 100.

To determine whether CaCF-induced suppression was antigen specific, mice were tolerized i.v. with CaCF and immunized 7 days later at two sites at the base of the tail with CaCF-CFA, 200 μg of CneF-CFA (30), or 100 μg of PPD-CFA (30) or on the surface of the ear with 0.5% DNFB as previously described (34). Six days after immunization (day 13), mice receiving CaCF, CneF, or PPD were footpad challenged with 10 to 50 μg of the respective antigens (10, 30) while animals receiving DNFB were ear challenged with 0.2% DNFB (34). Footpad or ear swelling was measured 24 h later (day 14).

To determine whether suppression could be adoptively transferred to recipient mice, 7 days after tolerization with CaCF, spleen (1 × 10⁶) or lymph node (5 × 10⁶) cells collected from the donor mice were adoptively transferred to naive recipient mice at the time of immunization with CaCF-CFA. Controls consisted of mice that received no cells or mice that received the same number of lymph node or spleen cells collected from naive mice. Mice were footpad challenged with CaCF 6 days after immunization, and swelling responses were measured 24 h later.

**Effects of Candida-specific Th1 and suppressor T (Ts) cells on experimental *Candida* vaginitis.** Mice were immunized with CaCF-CFA or tolerized with CaCF as described above. Four days after immunization or tolerization, mice were treated with the first of weekly subcutaneous injections of 0.5 mg of estradiol valerate to induce the condition of pseudopregnancy. Preliminary experiments showed that levels of CaCF-mediated DTH reactivity or suppression of DTH reactivity were similar in mice treated with and without exogenous estrogen. Seven days after immunization or tolerization, mice were given a vaginal inoculation of *C. albicans* (5 × 10⁵ blastoconidia per mouse) as previously described (10). DTH reactivity (footpad swelling following challenge with CaCF), vaginal *Candida* burden (vaginal lavage cultures), and wet-mount slide preparations of lavage fluid from separate mice were monitored weekly for 4 weeks as previously described (10). The amounts of hyphae on wet-mount slides were scored by a blinded observer by using a scale of 0 to ++++, where ++++ represented large sheets of hyphae and 0 referred to the absence of hyphae. In specific experiments, 1 × 10⁸ spleen cells or 5 × 10⁷ lymph node cells from immunized or tolerized mice were transferred to estrogen-treated recipient mice at the time of vaginal inoculation. The vaginal inoculum in these experiments was given in either 20 μl of PBS or 20 μl of CaCF. DTH and vaginal *Candida* burden were monitored for 4 weeks as described above. Also, in specific experiments, estradiol valerate was either not given or given in weekly doses of 0.1 mg per mouse instead of the usual 0.5 mg per mouse.

**Statistical analysis.** The unpaired Student t test was used to analyze the data. Significant differences were defined as a confidence level where *P* < 0.05 by a one-tailed test.

**RESULTS**

**Spleen cells from *Candida*-immunized mice respond to *Candida* antigens with Th1-type reactivity.** To assess the effects of preinduced CMI reactivity on experimental vaginal candidiasis, we first asked whether the CD4⁺ *Th* cells in CaCF-CFA-immunized mice (10) had in vitro reactivity similar to the Th1-type reactivity characteristic of mice with experimental vaginal candidiasis (11). For this, we assessed the ability of spleen cells from mice immunized with CaCF-CFA 7 days earlier to produce Th1/Th2-type lymphokines in vitro in response to *Candida* antigens. Responses from immunized mice were compared with those from naive mice. The results are summarized in Table 1. In unstimulated cultures, spleen cells from CaCF-immune mice produced significantly higher concentrations of IL-2 than those from naive mice (70.6 pg/ml versus 16.8 pg/ml, respectively; *P* < 0.05). These results were supported by high background levels of [³H]thymidine incorporation in unstimulated spleen cells from immunized but not from naive mice (data not shown). Culture of the immune cells with HKB or SCS did not result in detectable increases in IL-2. These results did not change with shorter or longer culture periods (24 and 72 h) or with the length of immunization (4 to 5 or 11 to 14 days) (data not shown).

Low levels of IFN-γ were detected in culture supernatants from unstimulated spleen cells collected from CaCF-CFA-immunized and naive mice (<1,000 pg/ml). Spleen cells from
immunized mice cultured with HKB or SCS showed increased concentrations of IFN-γ (21,521 and 2,812 pg/ml, respectively; P < 0.0005 and P < 0.01), whereas IFN-γ concentrations in supernatants from naive cells cultured with HKB or SCS remained low (Table 1).

Immune or naive spleen cell culture supernatants contained low concentrations of IL-4 (<10 pg/ml) under both unstimulated and Candida antigen-stimulated conditions (Table 1). Spleen cells from both immunized and naive mice did, however, have the ability to produce IL-4, since supernatants from concanavalin A-stimulated spleen cells had 290 and 150 pg of IL-4 per ml, respectively.

Taken together, these results indicate that systemic CMI in CaCF-immunized mice is characterized as Th1 type by virtue of DTH reactivity (10) and the production of IFN-γ, but not IL-4, by spleen cells in response to Candida antigens. The presence of IL-2 in cultures of spleen cells from immunized but not naive mice is also indicative of Th1-type reactivity. The lack of increased IL-2 detected following antigenic stimulation, together with high background levels of proliferation in unstimulated cultures, indicated that the activation of cells by in vivo immunization resulted in either maximal production or rapid utilization of IL-2.

Demonstration of Candida-specific Ts cells in mice injected i.v. with CaCF. To study experimental vaginal candidiasis in the presence of down-regulated Candida-specific CMI reactivity, we examined the potential of CaCF to induce Candida-specific Ts cells. For these experiments, CaCF was injected i.v. into mice 7 days prior to immunization with CaCF-CFA. The mice were footpad challenged 6 days after immunization. Footpad swelling responses 24 h later are shown in Table 2. Mice immunized subcutaneously with CaCF-CFA (group 2) showed significant DTH reactivity in comparison with negative-control mice (group 1). In mice tolerized with CaCF (group 4), DTH reactivity was suppressed 100%. In contrast, mice that received an i.v. injection of culture filtrate medium alone had reactions similar to those of the positive-control mice (group 3).

To determine whether the suppression induced by CaCF was specific to C. albicans, mice tolerized with CaCF were immunized with a variety of different antigens, including CneF, PPB, and DNFb, and the DTH reactivity to those particular antigens was assessed. The results are illustrated in Table 3. Tolerization of mice with CaCF resulted in 74% suppression of CaCF-induced DTH responses (group 3 compared with group 2; P < 0.0005) but did not suppress responses induced by CneF (group 7 compared with group 5), PPB (group 10 compared with group 9), or DNFb (group 13 compared with group 12). Tolerization of mice with CneF resulted in 73% suppression of CneF-induced DTH responses (group 6 compared with group 5; P < 0.00005), consistent with the induction of Ts cells by CneF in the murine model of cryptococcosis (30). Interestingly, mice tolerized with CaCF and immunized with CneF or DNFb had enhanced DTH reactivity in comparison with that of the respective positive-control mice. Thus, although antigen-specific suppression is evident by tolerization with CaCF, it appears that CaCF can enhance responsiveness to some irrelevant antigens by mechanisms which are not understood.

To confirm that CaCF was inducing Ts cells in mice that suppressed DTH reactivity, spleen or lymph node cells from tolerized donor mice were adoptively transferred to naive recipient mice at the time of CaCF-CFA immunization and

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TABLE 1. Lymphokine production by spleen cells from mice immunized with CaCF-CFA in response to Candida antigens

<table>
<thead>
<tr>
<th>Cell type</th>
<th>IL-2</th>
<th>IFN-γ</th>
<th>IL-4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No stimulus</td>
<td>HKB</td>
<td>SCS</td>
</tr>
<tr>
<td>Immune*</td>
<td>70.6 (11.1)*</td>
<td>60.5 (7.5)</td>
<td>62.2 (18.5)</td>
</tr>
<tr>
<td>Naive</td>
<td>16.8 (2.8)</td>
<td>8.6 (1.4)</td>
<td>12.4 (1.2)</td>
</tr>
</tbody>
</table>

* The table shows a summary of results from three experiments. Asterisks indicate where significant differences were found in immunized mice either by comparison with naive mice and/or in response to Candida antigens.

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TABLE 2. Suppression of DTH in mice injected i.v. with CaCF

<table>
<thead>
<tr>
<th>Group</th>
<th>Tolerizing Ag&lt;sup&gt;a&lt;/sup&gt; day 0</th>
<th>Immunizing Ag&lt;sup&gt;b&lt;/sup&gt; day 7</th>
<th>Mean increase in footpad thickness [mm] + SEM day 14</th>
<th>% Suppression</th>
<th>P&lt; to group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>--</td>
<td>CaCF</td>
<td>--</td>
<td>--</td>
<td>1. 0.0005</td>
</tr>
<tr>
<td>3</td>
<td>CFmed</td>
<td>CaCF</td>
<td>--</td>
<td>--</td>
<td>1. NS</td>
</tr>
<tr>
<td>4</td>
<td>CaCF</td>
<td>CaCF</td>
<td>--</td>
<td>100</td>
<td>3. 0.0005</td>
</tr>
</tbody>
</table>

<sup>a</sup> CFmed (culture filtrate medium) or CaCF was injected i.v.
<sup>b</sup> CaCF-CFA was injected subcutaneously.
<sup>c</sup> n=4; mice were footpad challenged with 10 μg (50 μl) of CaCF on day 13. Data are from one of three similar experiments.
DTH reactivity was determined 7 days later. Results showed that tolerized spleen or lymph node cells transferred to naive recipient mice reduced the DTH reactivities 52 and 73%, respectively \((P < 0.05 \text{ and } P < 0.025)\), compared with responses in CaCF-immunized positive-control mice (data not shown). Conversely, the transfer of naive lymph node or spleen cells did not significantly reduce the DTH reactivity in immunized recipient mice.

**Effects of Candida-specific T\(_{DH}\) and Ts cells on experimental Candida vaginitis.** To determine whether systemic Candida-specific CMI detectable with CaCF had an effect on the vaginal Candida burden and DTH reactivity upon subsequent vaginal infection, CaCF-CFA-immunized or CaCF-tolerized estrogenized mice were inoculated with viable \(C.\) albicans. Positive-control mice included estrogen-treated naive mice given a vaginal inoculum of \(C.\) albicans and estrogen-treated mice given PBS in the vagina. The resulting DTH reactivity and vaginal Candida burden through 4 weeks after vaginal inoculation are shown in Fig. 1. At the time of vaginal inoculation, DTH reactivity in immunized estrogen-treated mice was significantly increased over reactivity in estrogen-treated naive mice (Fig. 1A; \(P < 0.0005\)). Tolerized estrogen-treated mice containing Candida-specific Ts cells had negligible DTH reactivity at the time of vaginal inoculation. The presence of Candida-specific Ts cells in infected mice significantly suppressed the generation of DTH through the 4-week period compared with that in naive infected mice (Fig. 1A; \(P < 0.0001\)). In contrast, immunized infected mice had DTH reactivity equivalent to that of naive infected mice in weeks 2 through 4 after vaginal inoculation. Estrogen-treated mice given PBS in the vagina had negligible DTH reactivity and did not acquire a vaginal infection throughout the 4-week period (Fig. 1). Although the presence of Candida-specific Ts cells suppressed the infection-derived generation of systemic CMI and positive DTH reactivity was evident in immunized mice at the time of vaginal inoculation, vaginal Candida burdens were similar in preimmunized, pretolerized, and naive infected mice throughout the 4-week period (Fig. 1B). Wet-mount slide preparations of lavage fluid showed that the vaginas of preimmunized, pretolerized, and naive infected mice had similar amounts of hyphae, with scores ranging from ++ to +++ in weeks 1 through 3 and + to ++ in week 4.

To determine whether the lack of effect of Ts or T\(_{DH}\) cells on the vaginal Candida burden was due to lack of effective cellular circulation, a series of adoptive transfer experiments was performed. For these studies, lymph node or spleen cells from CaCF-immunized or -tolerized donor mice were transferred respectively to preimmunized or pretolerized estrogen-treated recipient mice at the time of vaginal inoculation. In selected mice, CaCF was used as the diluent in the vaginal inoculum instead of PBS in an attempt to traffic cells into the vagina, similar in principle to footpad challenge. Results of DTH reactivity and the vaginal Candida burden through 4 weeks after vaginal inoculation were compared with those obtained with mice that received naive spleen or lymph node cells. Despite 50% suppression of DTH reactivity throughout the infection in pretolerized recipient mice, and positive DTH reactivity prior to vaginal inoculation in preimmunized recip-

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**TABLE 3. Specificity of suppression by CaCF**

<table>
<thead>
<tr>
<th>Group</th>
<th>Tolerizing Ag d0</th>
<th>Immunizing Ag d7</th>
<th>Challenge Ag d3</th>
<th>Mean increase in footpad(\text{or ear}) thickness (mm) + SEM</th>
<th>% suppression</th>
<th>(p) compared to immune control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>—</td>
<td>—</td>
<td>CaCF</td>
<td>n</td>
<td>0.01 0.2 0.3 0.4 0.5 0.6</td>
<td>74</td>
</tr>
<tr>
<td>2</td>
<td>—</td>
<td>CaCF-CFA</td>
<td>CaCF</td>
<td>n</td>
<td>0.01 0.2 0.3 0.4 0.5 0.6</td>
<td>74</td>
</tr>
<tr>
<td>3</td>
<td>CaCF</td>
<td>CaCF-CFA</td>
<td>CaCF</td>
<td>n</td>
<td>0.01 0.2 0.3 0.4 0.5 0.6</td>
<td>74</td>
</tr>
<tr>
<td>4</td>
<td>—</td>
<td>—</td>
<td>CneF</td>
<td>n</td>
<td>0.01 0.2 0.3 0.4 0.5 0.6</td>
<td>74</td>
</tr>
<tr>
<td>5</td>
<td>—</td>
<td>CneF-CFA</td>
<td>CneF</td>
<td>n</td>
<td>0.01 0.2 0.3 0.4 0.5 0.6</td>
<td>74</td>
</tr>
<tr>
<td>6</td>
<td>CneF</td>
<td>CneF-CFA</td>
<td>CneF</td>
<td>n</td>
<td>0.01 0.2 0.3 0.4 0.5 0.6</td>
<td>74</td>
</tr>
<tr>
<td>7</td>
<td>CaCF</td>
<td>CneF-CFA</td>
<td>CneF</td>
<td>n</td>
<td>0.01 0.2 0.3 0.4 0.5 0.6</td>
<td>74</td>
</tr>
<tr>
<td>8</td>
<td>—</td>
<td>—</td>
<td>PPD</td>
<td>n</td>
<td>0.01 0.2 0.3 0.4 0.5 0.6</td>
<td>74</td>
</tr>
<tr>
<td>9</td>
<td>—</td>
<td>PPD-CFA</td>
<td>PPD</td>
<td>n</td>
<td>0.01 0.2 0.3 0.4 0.5 0.6</td>
<td>74</td>
</tr>
<tr>
<td>10</td>
<td>CaCF</td>
<td>PPD-CFA</td>
<td>PPD</td>
<td>n</td>
<td>0.01 0.2 0.3 0.4 0.5 0.6</td>
<td>74</td>
</tr>
<tr>
<td>11</td>
<td>—</td>
<td>—</td>
<td>DNFB</td>
<td>n</td>
<td>0.01 0.2 0.3 0.4 0.5 0.6</td>
<td>74</td>
</tr>
<tr>
<td>12</td>
<td>—</td>
<td>DNFB</td>
<td>DNFB</td>
<td>n</td>
<td>0.01 0.2 0.3 0.4 0.5 0.6</td>
<td>74</td>
</tr>
<tr>
<td>13</td>
<td>CaCF</td>
<td>DNFB</td>
<td>DNFB</td>
<td>n</td>
<td>0.01 0.2 0.3 0.4 0.5 0.6</td>
<td>74</td>
</tr>
</tbody>
</table>

\(a\) Footpad thickness measurements are shown for groups 1 through 10.

\(b\) Ear thickness measurements are shown for groups 11 through 13.

\(c\) \(n = 4\); data are from one of three similar experiments.
of before, although mice. exogenous estrogen not different from burdens. (time

immunized infected (imm-inf), tolerized infected (tol-inf), naive infected (Naive-inf), and control (Naive-PBS) mice were monitored for 4 weeks for DTH responses (A) and vaginal Candida burden (B). Separate groups of four mice were used at each time point. The mean footpad swelling (DTH) response values (A) and CFU (B) ± the standard errors of the mean (error bars) from three experiments are shown. Asterisks indicate where significant differences were found.

FIG. 1. Effect of preinduced Candida-specific T
DH and Ts cells on experimental vaginal candidiasis. Seven days after groups of mice were immunized with CaCF-CFA, tolerized with CaCF, or left untreated (naive), the mice were inoculated in the vagina with C. albicans. Control mice consisted of naive mice given PBS in the vagina. Weekly estrogen treatments began 72 h prior to vaginal inoculation. Immunized infected (imm-inf), tolerized infected (tol-inf), naive infected (Naive-inf), and control (Naive-PBS) mice were monitored for 4 weeks for DTH responses (A) and vaginal Candida burden (B). Separate groups of four mice were used at each time point. The mean footpad swelling (DTH) response values (A) and CFU (B) ± the standard errors of the mean (error bars) from three experiments are shown. Asterisks indicate where significant differences were found.

4.1 × 10⁴ CFU; P > 0.05). In another set of experiments, similar treatments were performed in nonestrogenized mice that spontaneously clear vaginal Candida infections within 2 to 3 weeks (11). Results collected through 3 weeks after vaginal inoculation showed that although DTH reactivity was suppressed (62%) in pretolerized mice throughout the infection and was positive in preimmunized mice prior to inoculation, the majority of mice in each group (>70%) cleared the vaginal infection by the second week, similar to nonestrogenized naive infected mice (data not shown).

**DISCUSSION**

There are considerable data to suggest that CMI is an important host defense mechanism for mucosal surfaces. Evidence for this comes from patients with chronic mucocutaneous candidiasis who characteristically lack both delayed cutaneous skin test reactivity and in vitro peripheral lymphocyte responsiveness to Candida antigens (1, 14, 18, 33). Using animal models, numerous investigators have demonstrated a role for CD4⁺ T cells in resistance to Candida infections of the skin or gastrointestinal tract (2, 5–8, 25) and a role for CD8⁺ T cells in the down-regulation of Candida-specific CMI (17). However, there is a paucity of information on the effects of peripheral CMI at the vaginal mucosa. This has largely been due to the lack of workable animal models of vaginal candidiasis in which the effects of CMI can be investigated. Recently, Cantorna et al. employed immunodeficient mice to examine vaginal candidiasis and found that susceptibility to natural vaginal infection was not increased in mice deficient in T or phagocytic cells compared with immunocompetent mice (4). However, these results are difficult to interpret with respect to our study, since mice were not maintained in pseudoeudra during the infection, a condition required to achieve optimal levels of infectivity in rodents (37, 40).

We recently reported that estrogenized mice given a vaginal inoculum of C. albicans acquire a persistent vaginal infection and develop Th1-type (3, 29) Candida-specific systemic CMI (10, 11), which has been reported to be associated with resistance to Candida infections (36). However, we observed that although Th1-type Candida-specific CMI peaked within 2 to 3 weeks after vaginal inoculation (11), the vaginal infection, quantitated by vaginal lavage culture and qualitized by the presence of hyphae in lavage fluid, persisted for up to 10 weeks (10). The objective of the present study was to examine the effects of preinduced Candida-specific peripheral CMI on experimental vaginal candidiasis.

In the present study, the positive in vivo DTH reactivity and in vitro spleen cell-mediated Th1-type lymphokine production (presence of IL-2 and IFN-γ but not IL-4) in immunized but not naive mice verified the presence of Candida-specific T
DH cells in CaCF-immunized animals at the time of vaginal inoculation. The Candida-specific suppression of immunization-induced DTH reactivity, and the fact that suppression could be adoptively transferred to recipient mice and additionally abrogated by in vivo antibody-mediated depletion of T cells (unpublished observations), indicated that CaCF could also be used to induce Candida-specific Ts cells. We reasoned that Candida-specific Ts cells would be a useful tool to study vaginal candidiasis under reduced Candida-specific CMI. The Ts cell approach was also relevant since Ts cells characterized both in patients with chronic mucocutaneous candidiasis (9, 35) and in murine models of cutaneous candidiasis (8, 17) have been implicated in the development of Candida infections. Indeed, the presence of Candida-specific Ts cells at the time of vaginal inoculation effectively reduced the subsequent vaginal

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infection-derived systemic CMI. However, irrespective of their effects on systemic CMI, Candida-specific T_{DLH} or Ts cells appeared to have no effect on vaginal Candida infection, as evidenced by the lack of differences in vaginal lavage cultures and relative amounts of hyphae in the lavage fluid within each group of infected mice. Results from a number of additional experiments eliminated potential causes, such as the lack of cellular circulation or the strong influence of exogenous estrogen, for the lack of effects by T_{DLH} or Ts cells on the vaginal Candida burden. Thus, our data indicate that preinduced peripheral CMI cannot effectively reduce or exacerbate the vaginal Candida burden during experimental vaginal candidiasis.

There are several possible explanations for the observed results. One possibility is that the Candida-specific Th1-type reactivity induced by systemic immunization by CaCF is functionally distinct from the peripheral Th1-type reactivity generated by vaginal infection. A similar scenario has been described for animals infected by Listeria monocytogenes (26, 27). L. monocytogenes-specific T_{DLH} cells induced in mice by immunization with killed L. monocytogenes in CFA were not protective against a Listeria infection, whereas T cells induced by immunization with viable L. monocytogenes promoted both DTH reactivity and protection against Listeria infection (26, 27).

Similarly, Domer et al. demonstrated that mannan-induced Th1 cells suppressed DTH responses induced by mannan but not those induced by Candida antigens containing no mannan (8). In our study, care was taken to employ a multiantigen culture filtrate preparation that potentially consisted of a broad spectrum of antigens capable of inducing many types of responses, including those which are immunoprotective. Furthermore, CaCF-induced Ts cells were capable of suppressing both CaCF- and C. albicans vaginal infection-induced DTH activities. However, until immunoprotective antigens are identified in CaCF, this explanation involving functionally distinct Th1-type CD4 cells cannot be eliminated. Studies to examine (i) whether Candida-specific T_{DLH} or Ts cells can protect or exacerbate gastrointestinal or systemic candidiasis and (ii) whether the Th1-type systemic CMI generated by a primary vaginal infection in nonestrogenized mice (11) is protective against a second vaginal infection in the presence of estrogen will likely provide additional insight into this important question.

A second possible explanation for the observed results is that CMI is not an important host defense mechanism against vaginal infections by C. albicans. This is unlikely, since there is considerable evidence suggesting a role for CMI at mucosal surfaces and additionally against Candida infections of the gastrointestinal tract (2, 5–8, 25).

A third explanation is that CMI expressed in the periphery does not represent a predominant host defense mechanism at the vaginal mucosa but that local expression of CMI in the vaginal mucosa is important for host defense against vaginal candidiasis. Indeed, recent studies have shown that T cells are present in the vaginal mucosa and that these vaginal T cells are phenotypically distinct from peripheral T cells (31). Thus, the vaginal mucosa may possess unique tissue-specific CMI with little trafficking of cells from the periphery. This hypothesis is supported by a number of clinical studies showing normal peripheral CMI reactivity in patients with RVVC during symptomatic attacks (12, 16, 41). This is particularly interesting in light of the fact that women with RVVC are not susceptible to oral or esophageal candidiasis, from which protection is presumed to be governed by peripheral CMI. Therefore, in addition to studies that more closely examine the role of peripheral CMI in the vaginal mucosa, studies to investigate the role of vaginal T cells as a host defense mechanism against vaginal candidiasis should also be undertaken. It appears that the experimental murine model of vaginal candidiasis is an excellent model for exploration of this important area of investigation.


