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Candida-Specific Th1-Type Responsiveness in Mice with Experimental Vaginal Candidiasis

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The role of systemic cell-mediated immunity (CMI) as a host defense mechanism in the vagina is poorly understood. Using a murine pseudoestrus model of experimental vaginal candidiasis, we previously found that animals given a vaginal inoculum of viable *Candida albicans* blastoconidia acquired a persistent vaginal infection and developed *Candida*-specific delayed-type hypersensitivity (DTH) responses. The present study was designed to characterize the peripheral CMI reactivity generated from the vaginal infection in mice and to determine whether pseudoestrus is a prerequisite for the induction of peripheral CMI reactivity. Mice treated or not treated with estrogen and given a vaginal inoculum of *C. albicans* blastoconidia were examined for 4 weeks for their vaginal *Candida* burden and peripheral CMI reactivity, including DTH responsiveness and in vitro Th1 (interleukin-2 [IL-2], gamma interferon [IFN-γ])/Th2 (IL-4, IL-10)-type lymphokine production in response to *Candida* antigens. Results showed that although mice not treated with estrogen before being given a vaginal inoculum of *C. albicans* blastoconidia developed only a short-lived vaginal infection and harbored significantly fewer *Candida* CFU in the vagina compared with those given estrogen and then infected; DTH reactivity was equivalent in both groups. In vitro measurement of CMI reactivity further showed that lymph node cells from both estrogen- and non-estrogen-treated infected mice produced elevated levels of IL-2 and IFN-γ in response to *Candida* antigens during the 4 weeks after vaginal inoculation. In contrast, lymph node cells from the same vaginally infected mice showed no IL-10 production and only small elevations of IL-4 during week 4 of infection. These results suggest that mice with experimental vaginal candidiasis develop predominantly Th1-type *Candida*-specific peripheral CMI reactivity and that similar patterns of Th1-type reactivity occur in mice regardless of the persistence of infection and the estrogen status of the infected mice.

Recurrent vulvovaginal candidiasis (RVVC) is an opportunistic mucosal infection that affects up to 5% of otherwise healthy women of childbearing age (17–19) and is frequently caused by *Candida* species (33). *Candida albicans* is the causative agent in approximately 85 to 90% of symptomatic, culture-positive RVVC patients (10, 17, 22, 24). Antimycotic agents, although effective in eradicating individual attacks, do not prevent recurrence in women with chronic or recurrent vaginitis. In contrast to women with infrequent episodes of vaginitis precipitated by pregnancy, oral contraceptives, uncontrolled diabetes mellitus, and particularly by the use of antibiotics, there are no recognized exogenous predisposing factors for RVVC (32). RVVC is presumed to result from diminished host defense mechanisms that increase susceptibility to symptomatic infection. Accordingly, it is postulated that the conversion of *C. albicans* from vaginal commensal to pathogen results from abnormalities in systemic cell-mediated immunity (CMI) similar to those associated with chronic mucocutaneous candidiasis (1, 4, 6, 9, 13, 25, 27, 37).

Clinical studies that have examined systemic CMI in patients with RVVC have produced somewhat controversial results (11, 15, 35, 38, 39). Although there is general agreement that most symptomatic, culture-positive RVVC patients have reduced delayed cutaneous skin test reactivity to *Candida* antigens (11, 35), results of studies of in vitro proliferation of peripheral blood mononuclear cells have been inconsistent. Some investigators report reduced responses to *Candida* antigens (15, 38, 39), whereas others describe normal responsiveness (11, 35). These conflicting data, together with the lack of well-defined animal models to study CMI during a vaginal infection, have hampered efforts to define the role of systemic CMI as a natural defense mechanism in the lower genital tract.

We recently used an estrogen-dependent murine model of experimental vaginal candidiasis to study CMI during a vaginal infection (7). These studies confirmed that estrogen-treated mice given a vaginal inoculum of *C. albicans* acquired a persistent vaginal infection. Furthermore, we showed that infected mice developed *Candida*-specific delayed-type hypersensitivity (DTH) responses that were indistinguishable from DTH reactivity induced in mice by systemic immunization with *Candida* antigens. The present study was designed to further characterize the peripheral CMI generated from the vaginal mucosa of infected mice and to determine whether pseudoestrus was a prerequisite for the induction of CMI reactivity. Mice treated with or without estrogen and given a vaginal inoculum of *C. albicans* blastoconidia were examined for 4 weeks for their vaginal *Candida* burden and peripheral CMI reactivity, including DTH responsiveness and *in vitro* Th1/Th2-type lymphokine production (3, 8, 23) in response to *Candida* antigens.

MATERIALS AND METHODS

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

Antigens. *C. albicans* culture filtrate antigen (CaCF) was prepared as previously described (7). *C. albicans* 3153A was grown for 3 days in a <12,000-molecular-weight dialysate

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medium prepared from 50% phytone-peptone and supplemented with 1% glucose. Supernatants from the 3-day culture were concentrated 10-fold on a 10,000-molecular-weight exclusion membrane (Amicon Corp., Danvers, Mass.) while being washed 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 Lowry 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. *C. albicans* heat-killed blastoconidia (HKB) were prepared by incubating a stationary-phase culture of *C. albicans* 3153A for 2 h at 60°C and washing it twice with PBS. *C. albicans* soluble cytoplasmic substances (SCS) (5) were a kind gift of Judith Domer, Tulane University School of Medicine, New Orleans, La.

Vaginal infection and DTH reactivity. Mice were infected as previously described (7). Briefly, 72 h prior to vaginal inoculation and weekly thereafter, mice were injected subcutaneously with 0.5 mg of estrodiol valerate (E. R. Squibb & Sons, Inc., Princeton, N.J.) dissolved in sesame oil. Control untreated mice received sesame oil alone. *C. albicans* 3153A blastoconidia (5×10^5) from a stationary-phase culture were inoculated in 20 μl of PBS into the vaginas of estrogen- and non-estrogen-treated mice. Control mice were treated with estrogen and given PBS in the vagina. At weekly intervals beginning 1 week after infection, groups of three or four mice were footpad challenged with 10 μg (50 μl) of CaCF as previously described (7) (one footpad received CaCF, and the contralateral pad received PBS). At 18 to 24 h later, the footpad swelling was measured with a micrometer and the mice were sacrificed. Vaginal lavages with 100 μl of PBS were performed on sacrificed mice, and the lavage fluid was plated onto Sabouraud dextrose agar as previously described (7). The 48- to 72-h lavage cultures were used to quantitate the vaginal *Candida* burden. Vaginal lavage fluid was also analyzed microscopically for the presence of hyphae by wet-mount slide preparations.

Preparation of LNC for in vitro CMI reactivity. Following collection of vaginal lavage fluid, lymph nodes (all) from sacrificed animals were collected and single-cell suspensions were prepared with a sterile mesh screen (preliminary experiments showed no difference in reactivity between the use of all lymph nodes and the use of only mesenteric, inguinal, and lumbar lymph nodes). The lymph node cells (LNC) were treated with Tris-ammonium chloride to lyse any erythrocytes, washed twice with Hanks balanced salt solution (GIBCO Laboratories, Grand Island, N.Y.), and resuspended in RPMI 1640 (GIBCO) supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), L-glutamine (2 mM), *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (20 mM) (all from GIBCO), β-mercaptoethanol (5×10^{-5} M) (Sigma), and 10% heat-inactivated fetal calf serum (GIBCO) (complete medium). Viable cells were counted in a hemacytometer by trypan blue dye exclusion.

Production of lymphokines. For lymphokine production, LNC were cultured at 4×10^6 cells per ml in 2.0 ml of complete medium alone (unstimulated) or with *C. albicans* HKB (5×10^6/ml) or *C. albicans* SCS (125 μg/ml). Cultures were incubated for 48 h at 37°C under 5% CO_2. Culture supernatants were collected by centrifugation and stored at −70°C.

Quantitation of IL-2. Culture supernatants were assayed for interleukin-2 (IL-2) content by using the IL-2-dependent cell line, CTLL-20 (American Type Culture Collection, Rockville, Md.) (14). The cells were maintained in complete medium supplemented with 5% concanavalin A-stimulated Lewis rat spleen cell supernatants. The supernatants were serially diluted in complete medium and added in triplicate to 96-well round-bottom tissue culture plates in a volume of 100 μl per well. CTLL-20 cells (5×10^4 cells per ml) were added to each well in a volume of 100 μl. After 16 h of culture, the wells were pulsed for 4 h with 1 μCi of [^{3}H]thymidine, and the radioactivity incorporated into the cells was collected onto fiberglass filters (type A/E; Gelman Sciences, Ann Arbor, Mich.) and counted in a liquid scintillation counter (Beckman Instruments, Irvine, Calif.). Human recombinant IL-2, kindly provided by Cetus Corp., Emeryville, Calif., was used as the standard and was serially diluted from 7,640 pg/ml (20 U/ml) to quantitate the amount of IL-2 present in the culture supernatants. A 70 to 80% abrogation of the antigen-stimulated supernatant-mediated proliferation of CTLL-20 cells was achieved by the addition of anti-IL-2 monoclonal antibody produced by the S4B6 hybridoma (kindly provided by Timothy Mosmann, University of Alberta, Alberta, Canada), confirming that IL-2 in the culture supernatants was stimulating the CTLL-20 cells. Production of IL-2 is expressed as specific IL-2, determined by subtracting the amount of IL-2 present in unstimulated cultures from the IL-2 content of antigen-stimulated cultures.

Quantitation of IFN-γ. A specific and sensitive enzyme-linked immunosorbent assay (ELISA) (Genzyme Corp., Cambridge, Mass.) was used to determine the concentration of gamma interferon (IFN-γ) in the culture supernatants. Optical densities at 490 nm were read by using a Maxline microplate reader (Molecular Devices Corp., Menlo Park, Calif.) with Softmax data analysis software. The data were expressed as picograms per milliliter by using polynomial regression analysis based on a standard curve obtained with murine recombinant IFN-γ (rIFN-γ). The sensitivity of the ELISA assay for IFN-γ was 125 pg/ml.

Quantitation of IL-4. Culture supernatants were assayed with the IL-4-dependent indicator cell line, CT.4S (16), kindly provided by William Paul, National Institutes of Health, Bethesda, Md. CT.4S cells were maintained in complete medium supplemented with 250 U of murine recombinant IL-4 (rIL-4), kindly provided by Immunex Corp., Seattle, Wash. To quantitate IL-4, CT.4S cells (2.5×10^4/ml) in 100 μl of complete medium were added to 96-well flat-bottom microtiter plates and cultured with 100 μl of serially diluted culture supernatants. After 24 h of culture, the wells were pulsed for an additional 24 h with 1 μCi of [^{3}H]thymidine. The radioactivity incorporated into the cells was counted in a liquid scintillation counter. Murine rIL-4 serially diluted from 200 pg/ml (20 U/ml) was used to generate a standard curve from which the concentration of IL-4 in the culture supernatants was determined. A 75 to 85% abrogation of the supernatant-mediated CT.4S cell proliferation was achieved with the addition of anti-IL-4 monoclonal antibody produced by the 11B11 hybridoma (kindly provided by William Paul) confirming that IL-4 in the supernatants was stimulating the CT.4S cells.

Quantitation of IL-10. A specific and sensitive ELISA (Endogen Corp., Boston, Mass.) was used to determine the concentration of IL-10 in the culture supernatants. Optical densities were read at a wavelength of 490 nm, and data were expressed as units per milliliter based on a standard curve generated from murine rIL-10. The sensitivity of the IL-10 ELISA was 0.14 U/ml.

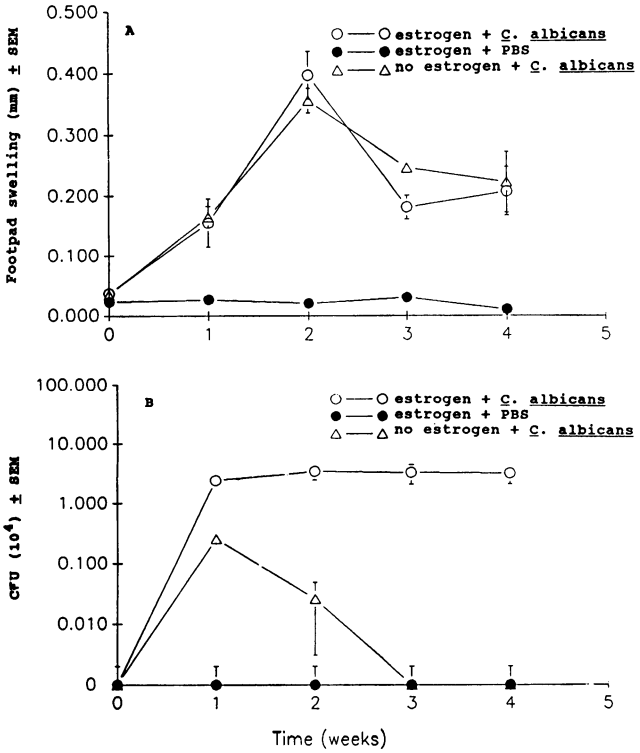

FIG. 1. Effects of pseudoestrus on vaginal infection and DTH reactivity in mice with experimental vaginal candidiasis. Groups of four mice were treated weekly with 0.5 mg of estrodiol valerate or sesame oil (no estrogen) beginning 72 h prior to vaginal inoculation. At weekly intervals, animals were tested for DTH reactivity 24 h after footpad challenge with CaCF (A) and vaginal lavages were performed on sacrificed mice to determine the vaginal *Candida* burden (B). Results are the means ± standard errors of the means from three experiments.

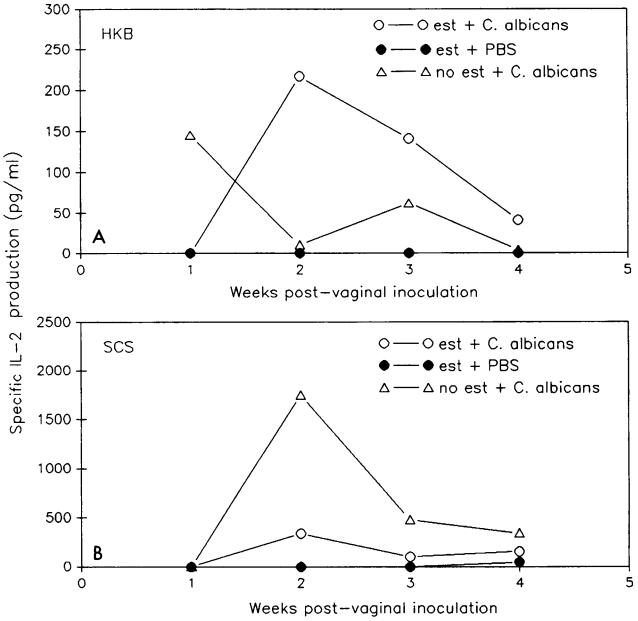

FIG. 2. IL-2 production in response to *Candida* antigens by LNC from vaginally infected mice. At weekly intervals following vaginal inoculation with *C. albicans* in the presence or absence of estrogen (est), LNC from groups of three mice were collected and cultured with *Candida* antigens, i.e., HKB (A) and SCS (B). The IL-2 content in 48-h culture supernatants was measured by proliferation of the IL-2-dependent cell line, CTLL-20, with human rIL-2 as the standard. Specific IL-2 production was calculated by subtracting the IL-2 level in unstimulated cultures from that in antigen-stimulated cultures. The average concentration of IL-2 in unstimulated cultures was 206 ± 93 pg/ml, and there were no differences between IL-2 in unstimulated cultures from infected and uninfected mice. The results shown are from a representative experiment of three repeats.

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

RESULTS

Vaginal *Candida* infection and resulting DTH reactivity. Estrogen- and non-estrogen-treated mice were inoculated in the vagina with 5×10^5 viable *C. albicans* blastoconidia. At weekly intervals for 4 weeks, the mice were footpad challenged with CaCF to determine *Candida*-specific DTH reactivity and vaginal lavages were performed to measure the vaginal *Candida* burden. The results are illustrated in Fig. 1. Estrogen-treated mice given a vaginal inoculum of *C. albicans* developed a high-titer vaginal infection (5×10^4 CFU/100 μl of lavage fluid), which persisted through the 4-week period (Fig. 1B). DTH reactivity in estrogen-treated infected mice was evident at 1 week postinoculation, peaked at 2 weeks postinoculation, and remained positive at 4 weeks (Fig. 1A). Mice given a vaginal inoculum and weekly injections of sesame oil without estrogen developed a low-titer vaginal infection with significantly fewer CFU of *C. albicans* (2.6×10^3 CFU/100 μl of lavage fluid) compared with the *Candida* burden in estrogen-treated infected mice ($P < 0.005$). The vaginal infection in non-estrogen-treated mice cleared by week 3 (Fig. 1B). However, DTH reactivity in non-estrogen-treated infected mice was not significantly different from that in estrogen-treated infected mice ($P > 0.05$) throughout the 4-week period (Fig. 1A). Estrogen-treated mice inoculated vaginally with PBS (i.e., uninfected) did not acquire a vaginal *Candida* infection or develop *Candida*-specific DTH reactivity.

In vitro CMI reactivity of LNC during vaginal *Candida* infection. LNC from estrogen-treated infected, estrogen-treated uninfected, and non-estrogen-treated infected mice were assessed for in vitro production of IL-2, IFN-γ, IL-4, and IL-10 in response to particulate and soluble *Candida* antigens.

(i) IL-2 production. LNC from estrogen- and non-estrogen-treated vaginally infected mice but not from estrogen-treated uninfected control mice produced detectable concentrations of IL-2 in response to *Candida* antigens during the 4 weeks after vaginal inoculation (Fig. 2). Peak levels of antigen-stimulated IL-2 occurred early during the infection (weeks 1 to 2) and declined to low levels by week 4. In response to *C. albicans* HKB (Fig. 2A), the amount of IL-2 produced by LNC from non-estrogen-treated mice peaked 1 week after vaginal inoculation (150 pg/ml) and oscillated between 0 and 50 pg/ml through week 4. In estrogen-treated mice, the LNC-mediated IL-2 production peaked at week 2 (220 pg/ml) and declined to less than 50 pg/ml by week 4. In response to SCS (Fig. 2B), peak levels of IL-2 in both estrogen- and non-estrogen-treated infected mice occurred at week 2 (400

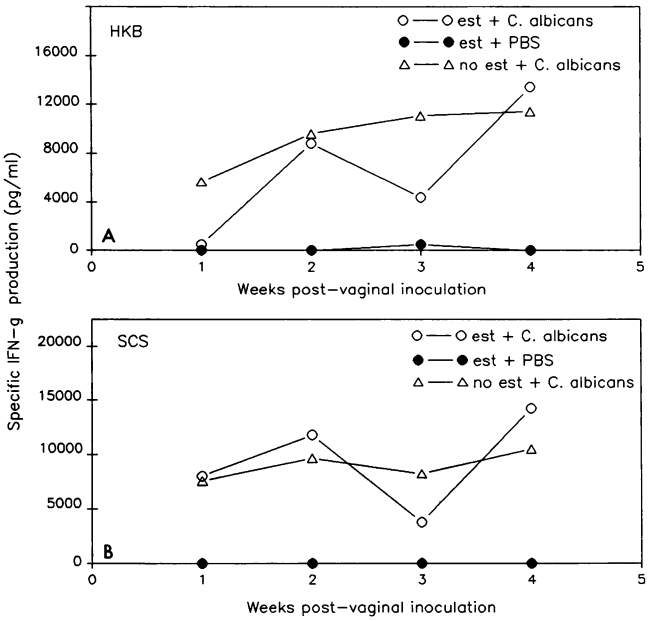

FIG. 3. IFN-γ production in response to *Candida* antigens by LNC from vaginally infected mice. At weekly intervals following vaginal inoculation with *C. albicans* in the presence or absence of estrogen (est), LNC from groups of three mice were collected and cultured with *Candida* antigens, i.e., HKB (A) and SCS (B). The IFN-γ content in 48-h culture supernatants was measured by a sensitive and specific ELISA for murine IFN-γ. Specific IFN-γ production was calculated by subtracting the IFN-γ level in unstimulated cultures from that in antigen-stimulated cultures. The average concentration of IFN-γ produced by LNC from infected mice (4,778 ± 2,091 pg/ml) was significantly different from that produced by LNC from uninfected mice (896 ± 1,000 pg/ml). The results shown are from a representative experiment of three repeats.

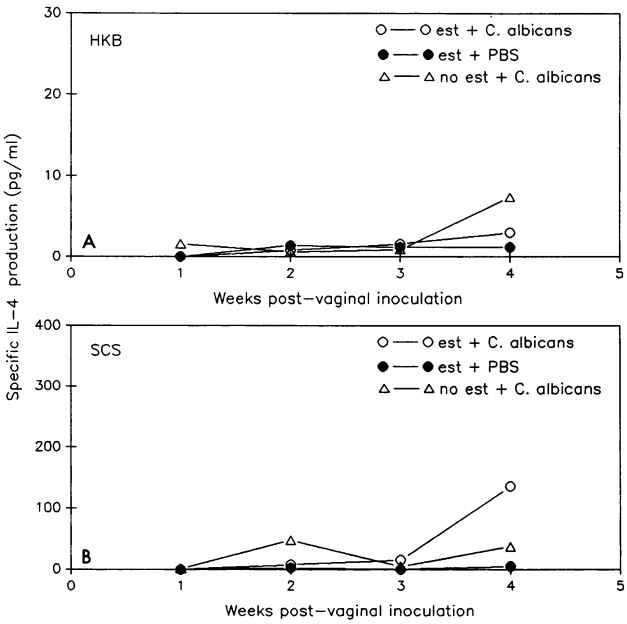

FIG. 4. IL-4 production in response to *Candida* antigens by LNC from vaginally infected mice. At weekly intervals following vaginal inoculation with *C. albicans* in the presence or absence of estrogen (est), LNC from groups of three mice were collected and cultured with *Candida* antigens, i.e., HKB (A) and SCS (B). The IL-4 content in 48-h culture supernatants was measured by the proliferation of the IL-4-dependent cell line CT.4S, with murine rIL-4 as the standard. Specific IL-4 production was calculated by subtracting the IL-4 level in unstimulated cultures from that in antigen-stimulated cultures. The average IL-4 concentration in unstimulated cultures was 4.38 ± 0.86 pg/ml, and there were no differences between the IL-4 levels in unstimulated cultures from infected and uninfected mice. The results shown are from a representative experiment of three repeats.

and 1,700 pg/ml, respectively) and the levels declined to 100 and 400 pg/ml, respectively, by week 4.

(ii) IFN-γ production. LNC from infected mice had significantly higher levels of IFN-γ in unstimulated cultures than did LNC from estrogen-treated uninfected control mice (4,778 ± 2,091 pg/ml and 896 ± 1,000 pg/ml, respectively). LNC from estrogen-treated, uninfected control mice did not produce IFN-γ in response to *Candida* antigens (Fig. 3). LNC from both estrogen- and non-estrogen-treated infected mice produced elevated concentrations of IFN-γ in response to *C. albicans* HKB (Fig. 3A) and SCS (Fig. 3B) throughout the 4 weeks after vaginal inoculation. There were no distinct differences in IFN-γ production between estrogen- and non-estrogen-treated infected mice.

(iii) IL-4 production. IL-4 production by LNC from vaginally infected mice in response to *Candida* antigens is shown in Fig. 4. Through the first 3 weeks of infection, negligible concentrations of IL-4 were observed in antigen-stimulated LNC cultures from both infected and uninfected mice. However, at week 4, IL-4 production by LNC in response to *C. albicans* HKB (Fig. 4A) and SCS (Fig. 4B) from both estrogen- and non-estrogen-treated infected mice increased to concentrations above those in uninfected control mice.

(iv) IL-10 production. Culture supernatants were also tested for the presence of IL-10. Throughout the observed infection period, negligible levels of IL-10 were present in LNC cultures from both infected and uninfected mice, either unstimulated or stimulated with *C. albicans* HKB or SCS. The only exception was 0.216 U of IL-10 per ml produced in response to SCS by LNC from estrogen-treated mice 1 week after vaginal inoculation (the sensitivity of the assay is 0.14 U/ml).

DISCUSSION

The role of systemic CMI as a host defense mechanism for the vaginal mucosa is not well understood, and progress has been hampered by the lack of studies with animal models. We recently reported that mice maintained on estrogen and given a vaginal inoculum of *C. albicans* acquired a long-term vaginal infection (>10 weeks) characterized by demonstrable *Candida*-specific peripheral DTH reactivity that was comparable to that in animals immunized with *Candida* antigens (7). The purpose of the present study was to further characterize murine peripheral CMI reactivity during experimental vaginal candidiasis and to determine whether pseudoestrus, which is required to sustain vaginal infections in rodents (31, 34), was a prerequisite for the induction of peripheral CMI reactivity. We report here the first study demonstrating that Th1-type CMI reactivity is induced in the periphery as a result of a localized mucosal *Candida* infection and the first study characterizing peripheral CMI reactivity generated from a vaginal infection.

Analysis of the specific lymphokines produced by LNC from infected mice showed that for 4 weeks after vaginal inoculation, concentrations of IL-2 and IFN-γ were elevated

in response to both *Candida* antigens. IL-2 was produced at high levels early (first 2 weeks) during the infection, and the levels declined thereafter. Furthermore, the presence of IL-2, a known growth factor for T cells, correlated with the proliferative activity in response to *Candida* antigens of LNC from the same infected mice (data not shown). The IFN-γ levels in antigen-stimulated cultures from infected mice were elevated throughout the 4-week period, even though the levels of IFN-γ in unstimulated cultures were higher than in cultures from uninfected control mice. In contrast to IL-2 and IFN-γ, detectable levels of IL-4 and IL-10 were not consistently observed in *Candida* antigen-stimulated cultures from infected mice during the first 3 weeks of infection, and only small increases in IL-4 levels were observed at week 4. These observations, together with the presence of DTH reactivity, indicate that peripheral CMI reactivity generated by vaginal *Candida* infection is predominantly of the Th1 type (3, 23) and suggests that antigens introduced by vaginal inoculation of *C. albicans* preferentially induce *Candida*-specific Th1-type (DTH, IL-2, IFN-γ) rather than Th2-type (IL-4, IL-10) $CD4^+$ T cells (3, 8, 23).

It has been shown that Th1- and Th2-associated cytokines have reciprocal effects on one another. Specifically, IFN-γ secreted by Th1 cells down-regulates certain functions of IL-4, i.e., B-cell activation (28) and induction of class II major histocompatibility complex on B cells (21), and can inhibit Th2 cell proliferation (12). Conversely, IL-10 produced by Th2 cells has been shown to inhibit lymphokine production and proliferation by Th1 cells (8). In the present study, it is unclear whether there is any regulatory activity between putative Th1 and Th2 cells or by the respective lymphokines they produce. However, the ability of LNC to produce consistently high levels of IFN-γ in response to *Candida* antigens throughout the 4 weeks after vaginal inoculation may be responsible for the low levels of antigen-stimulated Th2-type lymphokines (IL-4 and IL-10) observed during the same period. Examination of IL-2, IFN-γ, IL-4, and IL-10 production beyond 4 weeks of vaginal infection may provide additional evidence to confirm this potential Th1-associated lymphokine regulation.

In the present study, it was evident that the short-lived vaginal infection in non-estrogen-treated mice, which was characterized by significantly lower *Candida* population levels (Fig. 1), resulted in *Candida*-specific Th1-type reactivity that was similar to that in estrogen-treated infected mice. Furthermore, CMI reactivity in non-estrogen-treated mice was maintained following clearance of the vaginal infection (weeks 3 and 4). Therefore, it appears that *Candida* antigens present in a short-lived, low-grade infection are sufficient to generate and sustain peripheral CMI reactivity and that a lack of estrogen does not influence the ratio of Th1- to Th2-type reactivity during vaginal infection.

There is considerable evidence to suggest that Th1-type responses are associated with protection from or resistance to acute infection caused by many pathogens, including *Candida* species, whereas the presence of Th2-type responses has been observed during progressive infection (2, 26, 29, 36, 40). By using virulent and avirulent strains of *C. albicans* or a second system involving strains of mice that are susceptible or resistant to *Candida* infection, it has been shown that *Candida*-specific Th1 $CD4^+$ cells were associated with protection against systemic *Candida* infection whereas fatal disseminated *Candida* infection occurred in the presence of *Candida*-specific Th2 cells (2, 29). Furthermore, during systemic *Candida* infection, treatment of mice with anti-IL-4 antibodies resulted in a switch from Th2- to Th1-type responses concomitant with clearance of an otherwise fatal infection (30). From the results of this study, it is unclear whether peripheral Th1-type reactivity is protective against a vaginal *Candida* infection. It is possible, on the basis of the persistent presence of Th1-type reactivity, that *Candida* clearance from the vaginas of non-estrogen-treated mice was the result of Th1-type reactivity and was not simply due to a lack of exogenous estrogen, which provides a suitable environment for persistent growth of *Candida* species (31, 34). This interpretation supports a recent observation of Th1 cell-associated clearance of a primary respiratory tract infection with *Bordetella pertussis* in mice (20) and suggests that non-estrogen-treated mice can become immunized against *Candida* species by a short-term, low-grade vaginal infection. Alternatively, the lack of clearance of *Candida* organisms from the vaginas of estrogenized mice, in which a vaginal infection was sustained despite the presence of peripheral Th1-type reactivity, may indicate that peripheral Th1-type CMI reactivity either does not efficiently circulate to vaginal mucosal tissue under normal conditions or is simply not protective against a *Candida* vaginal infection. Experiments that examine vaginal infections in mice in the presence of preinduced Th1-type reactivity and that examine whether vaginally infected mice are protected from systemic or gastrointestinal candidiasis should shed considerable light on the role of *Candida*-specific peripheral Th1-type reactivity for mucosal surfaces of the vagina.

In summary, this study demonstrates that *Candida*-specific peripheral CMI reactivity generated during vaginal *Candida* infection is predominantly of the Th1 type, typified by high levels of peripheral LNC-mediated IL-2 and IFN-γ production in response to *Candida* antigens, together with low levels of the Th2-type lymphokines, IL-4 and IL-10. Furthermore, patterns of Th1-type peripheral CMI reactivity are similar in mice with a persistent or short-lived vaginal infection, raising interesting questions about the potential function of peripheral Th1-type reactivity at mucosal surfaces of the vagina. Results of these and future studies with animal models of vaginal candidiasis may be of value in determining immunological factors which predispose to recurrent vaginitis.

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