Genetic Basis for Protection against Experimental Vaginal Candidiasis by Peripheral Immunization

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In these studies, significant protection against experimental vaginal candidiasis after a subcutaneous immunization with Candida albicans extract was achieved in BALB/c mice but not in C57BL/6 (B6) mice. Protection from vaginal candidiasis was transferred to naive BALB/c mice by a population of spleen cells derived from immunized BALB/c mice. Removal of CD3 or CD4 but not CD8 T cells before transfer completely abrogated resistance to vaginal candidiasis. Recombinant inbred (RI) strains of mice derived from BALB/c and B6 strains were used for mapping loci that might be responsible for regulating vaginal protection after subcutaneous immunization. Linkage analysis using microsatellite-based genome mapping in these RI strains revealed four candidate loci on chromosomes 3, 7, 8, and 18 that exhibit statistically significant linkage to the strain distribution pattern. These results may contribute to the understanding of host genetic factors controlling the immune response to vaginal infections.

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The increase in sexually transmitted diseases and other vaginal infections throughout the world has increased the interest in generating vaginal immunity against an array of organisms, such as Candida albicans [1, 2], Trichomonas vaginalis [3], herpes simplex virus [4], and Chlamydia trachomatis [5], which can have dire pathogenic consequences. In addition to introduction of pathogens from exogenous sources such as sex partners, some vaginal infections such as candidiasis can occur when the organisms switch from the commensal to pathogenic state [6]. The immune cellular composition of the vaginal mucosa, as well as other mucosal surfaces, appears to be distinct and unique from that of other organs [7]. For example, previous work has shown that vaginal T cells expressing a γ/δ T cell receptor have several features that distinguish them from this subpopulation of T cells present in other tissues [8]. The role of the various sets of immune cells in protecting the vaginal mucosa from colonization by pathogenic organisms remains unknown or at best controversial.

C. albicans is a normal component of the vaginal flora of a moderate number of women; this organism can cause opportunistic infections presenting commonly either as episodic infections or, in some persons, as recurrent vulvovaginal candidiasis. Recurrent vulvovaginal candidiasis is a mucosal infection affecting ~5% of the female population who are otherwise healthy [9]. C. albicans is the pathogen in >85% of persons with recurrent vulvovaginal candidiasis [10]. It has been postulated that this syndrome is the result of a down-regulation of the cell-mediated immune response, which leaves the person susceptible to infection [11]. However, studies have shown that there is no statistical difference in either in vivo delayed-type hypersensitivity or in vitro peripheral blood lymphocyte T cell responses and humoral immunity between women with recurrent vulvovaginal candidiasis and asymptomatic women [1, 2]. Thus, the relationship between peripheral immunity and vaginal immunity with respect to recurrent vulvovaginal candidiasis patients remains unclear. The role and importance of T cells in preventing murine candidiasis has been demonstrated in resistance to oral and gut mucosal infections as well as systemic infections [13–15]. However, until recently, few studies have been done to determine the basis for immunity to vaginal candidiasis.

To facilitate the immunologic studies related to vaginal candidiasis, an estrogen-dependent murine model has been used [2]. With this murine model, previous work demonstrated that subcutaneous immunization that produces an effective systemic cell-mediated immunity does not correlate with protection against vaginal candidiasis in all strains of mice tested [16]. However, experimental data related to vaginal infections caused by the protozoan T. vaginalis indicates that subcutaneous immunization of BALB/c mice with this organism does generate a protective immune response against a vaginal infection with T. vaginalis [3]. It has recently been suggested that the response to infection and immunization protection could be associated with the route of immunization [5], resulting in organ-specific immune responses [3, 4]. Despite these facts, little is known about host defense factors that are important for protection against vaginal infections.

In the present study, the protective effects of subcutaneous immunization with C. albicans extract in a murine pseudoestrus model of experimental vaginal candidiasis were studied. Adoptive transfer studies were used to determine the immune cells that mediate resistance to vaginal candidiasis after peripheral
immunization. Furthermore, genetic studies using recombinant inbred (RI) strains of mice were done to identify candidate loci involved in controlling the immune response to vaginal candidiasis after peripheral immunization.

Materials and Methods

**Mice.** Female BALB/c ByJ, BALB/c J, C57BL/6 ByJ, and 12 C×B RI mice (8–10 weeks of age) were purchased from Jackson Laboratories (Bar Harbor, ME). Female BALB/c AnNCI mice were purchased from the National Cancer Institute (Bethesda, MD). The animals were housed in our animal facility colony at Allegheny University of the Health Sciences.

**Organism.** C. albicans B311 (ATCC 32354) was maintained at 23°C by weekly transfer on Sabouraud dextrose agar (SDA) plates. For all experiments, C. albicans was cultured into a fresh SDA plate 24 h before use and subsequently inoculated into 100 mL of medium (described in [17]). After incubation for 12 h at 25°C under constant agitation, yeast cells were harvested by centrifugation for 10 min at 5000 g at 4°C and washed three times in PBS. The final pellet was resuspended in PBS at 2.5 x 10^7 cells/mL.

**Antigen.** Crude cytoplasmic extract of C. albicans yeast was made as previously described [18]. Briefly, C. albicans was cultured into peptone-glucose medium, and the culture was incubated for 12 h at 25°C under constant agitation. The cell slurry harvested from the medium was mixed with an equal volume of glass beads and subjected to mechanical disruption in a Braun homogenizer for 8 min. Samples were examined microscopically for cell breakage. After beads were separated from cells, the supernatant pool was centrifuged at 12,000 g for 1 h at 4°C. The supernatant containing soluble cytoplasmic antigens was dialyzed and the protein concentration determined as 9.6 mg/mL. Mice were injected subcutaneously under the base of the tail with 100 µg of C. albicans extract (CaX) in complete Freund’s adjuvant (CFA) for immunization. The cytoplasmic extract was stored at −70°C until use.

**Estrogen treatment.** Three days before vaginal inoculation with C. albicans, mice received the first of weekly subcutaneous injections of 0.05 mL of estradiol valerate (Delestrogen; Squibb, Princeton, NJ; 0.5 mg subcutaneously) to induce pseudoestrus.

**Vaginal infection and collection of vaginal fluid.** Five weeks after mice were immunized subcutaneously with CaX, immunized and control mice were inoculated intravaginally with 5 x 10^5 viable yeast cells in 0.02 mL of PBS by use of an Eppendorf pipette. The vaginal samples were taken by washing the vaginal cavity by gentle aspiration of 0.05 mL of PBS, repeated four times with the same 0.05 mL and always done by the same operator. Vaginal washes were collected every 3 days after infection for data shown in figures 1 and 2 and on day 6 after infection for the adoptive transfer and genetic studies. A time course study demonstrated that days 3–6 after vaginal inoculation were the best in which to observe a difference in the number of Candida colonies obtained from vaginal lavage between immunized and immunized mice. The vaginal lavage content of each 0.05-mL sample was suspended in 0.5 mL of PBS and diluted serially; 0.1 mL quantities of these serial dilutions were inoculated onto plates containing prewarmed SDA supplemented with penicillin-streptomycin. The plates were then incubated for 48 h at 37°C, and the C. albicans colony-forming units (cfu) were counted.

**Immunosuppression.** Mice were immunosuppressed by a sublethal dose of irradiation (550 rad). Immunosuppression by this specific dose has been shown to abrogate primary immune responses [19, 20].

**In vitro depletion of T cell subsets: adoptive cell transfer.** Donor cells were derived from the spleens of BALB/c mice, which were subcutaneously immunized with 100 µg of CaX 5 weeks before transfer. Depletion of the donor splenic lymphocyte population was done by treatment of spleen cells with antibody and complement followed by two extra rounds of depletion. Depletion of T lymphocytes was done with anti-CD3 monoclonal antibody produced by YCD3 hybridoma cells. Depletion of the T cell subsets, CD4 and CD8, was done with GK1.5 and 2.43 monoclonal antibodies, respectively. Donor cells were incubated with antibody for 1 h at 4°C, followed by addition of 10% baby rabbit complement and incubation for 1 h at 37°C. After the three rounds of
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Effects of subcutaneous immunization on subsequent vaginal infection. Experiments were done to determine if a peripheral route of immunization could protect BALB/c mice against a subsequent vaginal C. albicans infection. Previous studies have demonstrated that vaginal inoculation of C. albicans in estrogen-treated mice results in infection characterized by invasion of vaginal tissue [2]. Histologic examination of vaginal sections from mice used in our studies confirmed the invasive nature of the infection (data not shown). Mice were immunized subcutaneously with CaX in CFA and, 5 weeks later, challenged intravaginally with viable C. albicans. Vaginal lavage was done at several time points after infection. Growth of C. albicans was compared between previously immunized mice and control unimmunized mice. In a typical experiment of three done with similar results (figure 1A), immunized BALB/c mice were significantly protected from vaginal infection compared with unimmunized mice, as determined by differences in the number of C. albicans cfu present in vaginal lavage samples (P < .05). In contrast to previous studies [16], these results clearly demonstrate that peripheral immunization with CaX protects BALB/c mice from a subsequent vaginal C. albicans challenge.

Figure 2. Effects of immunosuppression on ability of immunized BALB/c mice to clear experimental vaginal candidiasis. Five weeks after mice were immunized with C. albicans extract, they were immunosuppressed by sublethal irradiation (550 rad) and 2 days later inoculated intravaginally with C. albicans. This assay is representative of 2 done. Difference between unimmunized and immunized groups days 3, 6, and 14 after infection was significant (P < .05; Student’s t test on analysis of variance).

depletion, cells were washed three times in PBS. Depleted spleen cells contained <3% T cells as determined by flow cytometric analysis. Depleted or nondepleted spleen cells (10⁷) were injected intravenously into the tail vein of sublethally irradiated recipient mice. Recipient mice were inoculated intravaginally with viable C. albicans 1 week after the transfer of cells. On day 6 after infection, vaginal lavage samples were taken and enumerated as described above.

Linkage analysis. Genotypic strain distribution patterns for the C×B RI strains used for these experiments are available in the Mouse Genome Database. For the purpose of determining linkage in the RI strain sets, computer software (Map Manager QTLb11) was used.

Statistical analysis. The significance of differences in cfu was assessed by Student’s t test. Significant differences were defined as a confidence level at which P < .05 by a one-tailed test. Simple regression statistics were used for the genetic linkage analysis [21].

Results

Effects of subcutaneous immunization on subsequent vaginal infection. Experiments were done to determine if a peripheral route of immunization could protect BALB/c mice against a subsequent vaginal C. albicans infection. Previous studies have demonstrated that vaginal inoculation of C. albicans in estrogen-treated mice results in infection characterized by invasion of vaginal tissue [2]. Histologic examination of vaginal sections from mice used in our studies confirmed the invasive nature of the infection (data not shown). Mice were immunized subcutaneously with CaX in CFA and, 5 weeks later, challenged intravaginally with viable C. albicans. Vaginal lavage was done at several time points after infection. Growth of C. albicans was compared between previously immunized mice and control unimmunized mice. In a typical experiment of three done with
cans. This radiation treatment has been demonstrated to abrogate primary immune responses while leaving secondary immune responses intact [23, 24]. Vaginal lavage was done on subsequent days to determine fungal burden. Previously immunized BALB/c mice, which were immunosuppressed before vaginal challenge with C. albicans, showed a significant increase in their ability to clear the yeast infection compared with unimmunized mice (\(P < .05\) for days 3, 6, and 14 after infection; figure 2). Therefore, immunosuppression did not abrogate the protective effects of peripheral immunization in BALB/c mice, indicating that protection against vaginal candidiasis after peripheral immunization is mediated by a secondary immune response.

To determine if T cells play a role in protection against vaginal candidiasis after peripheral immunization, the potential of immunized BALB/c donor spleen cells, which were depleted of T cells, to transfer protection to unimmunized BALB/c recipient mice was examined. For these experiments, BALB/c mice were subcutaneously immunized with CaX in CFA. Five weeks later, spleen cells were harvested from these mice and depleted of CD3 T cells and CD4 or CD8 T cell subsets by complement-mediated cytoxicity by use of YCD3, GK1.5, or 2.43 antibodies, respectively. Cells were analyzed by flow cytometry to confirm cellular depletion of the appropriate population of cells. We have previously shown that spleen cells derived from BALB/c mice that have been immunized with this CaX are reactive to C. albicans antigens in a lymphocyte proliferation assay [15], and donor cells used in this experiment also demonstrated C. albicans–specific activity (data not shown). Depleted or nondepleted spleen cells were injected intravenously (\(10^7\) cells/mouse) into unimmunized, sublethally irradiated BALB/c mice. One week later, estrogen-treated recipient mice were inoculated with C. albicans intravaginally, and the vaginal C. albicans burden was quantitated on day 6 after infection.

Mice that were previously subcutaneously immunized with CaX in CFA had significantly (\(P < .01\)) lower C. albicans burden in vaginal lavage fluid than did unimmunized control mice (figure 3). Immunologically naive BALB/c mice that received spleen cells from immunized BALB/c mice also had significantly (\(P < .02\)) lower C. albicans burden in vaginal lavage fluids than did immunized control mice. Transfer of C. albicans–immune spleen cells depleted of either CD3 or CD4 T cells abrogated protection conferred by the immune cells to vaginal candidiasis (\(P < .01\) and \(P < .02\), respectively) (figure 3). In contrast, depletion of CD8 T cells from C. albicans–immune spleen cells before transfer did not affect the protection conferred by the immune cells. These results demonstrate an essential role of T cells and specifically CD4 T cells in protection against vaginal candidiasis after peripheral immunization in BALB/c mice.

Genetic analysis of the mechanisms of host resistance to vaginal candidiasis after peripheral immunization. Since the data described above clearly indicated that there are differences between BALB/c and B6 mice with respect to their ability to be protected against vaginal candidiasis after peripheral immunization, the ability of BALB/c mice to be protected after peripheral immunization appears to be genetically regulated. To determine if resistance to vaginal candidiasis after subcutaneous immunization is a dominant or recessive trait, subcutaneously immunized (BALB/c × C57BL/6)\(_F_1\) mice were examined for their ability to be protected against vaginal candidiasis. Unimmunized and immunized \(F_1\) mice were intravaginally challenged with viable C. albicans as previously described, and vaginal lavage was done on day 6 after infection. Immunized \(F_1\) mice had a significantly (\(P < .01\)) lower C. albicans burden than did unimmunized mice (figure 4). These results demonstrate that resistance to vaginal candidiasis after peripheral immunization is a dominant trait.

As a first step toward identifying candidate loci involved in controlling the immune response to vaginal candidiasis after peripheral immunization, 12 RI strains of mice derived from BALB/c and B6 (C×B RI) strains were phenotyped for their ability to be protected against vaginal infection with C. albicans after a peripheral immunization. Our previous experiments indicated that subcutaneously immunized BALB/c mice have an average of \(<75\) cfu of C. albicans in vaginal lavage fluids at day 6 after infection, whereas B6 mice have \(>100\) cfu of C. albicans. Therefore, the number of colonies obtained by vaginal lavage at day 6 after infection determined if an RI strain was...
Generations of backcross mice (BALB/c × C57BL/6) are currently being tested with these markers to confirm results in the C×B RI strains and locate with higher statistical significance the loci that control resistance to vaginal candidiasis after peripheral immunization.

Discussion

In these studies, the protective effects of subcutaneous immunization with C. albicans extract in a murine pseudoestrus model of experimental vaginal candidiasis were examined. BALB/c mice immunized subcutaneously with C. albicans failed to confer significant protection against vaginal candidiasis infection. These studies also show that the protection afforded by the subcutaneous immunization in BALB/c mice is not abrogated by immunosuppression with sublethal irradiation (550 rad), indicating that protection is mediated by a secondary immune response. The protection from vaginal challenge after peripheral immunization in BALB/c mice is associated with a CD4, but not CD8, population of T cells.

In contrast to the results demonstrated by the present studies, previous studies had demonstrated that peripheral immunization of a different strain of BALB/c mice, BALB/c J, failed to protect against vaginal challenge [16]. To determine whether this difference in substrains might account for the differences observed between these two strains, the substrains BALB/c ByJ, BALB/c-like (resistant), and was typed as c, or B6-like (susceptible), and was typed as b. Simultaneously, groups of unimmunized and subcutaneously immunized BALB/c and B6 mice were treated exactly the same way as the RI strains to serve as controls. The phenotypes of the 12 C×B RI strains and the strain distribution patterns obtained are shown in figure 5. Phenotyping of these 12 C×B RI strains and 90 backcross mice (susceptible, 28; resistant, 62; ratio, 1:2.2) suggested that resistance to vaginal candidiasis after subcutaneous immunization is controlled by at least one genetic locus, but most likely two loci are involved.

The strain distribution pattern for protection against vaginal candidiasis after peripheral immunization was compared with the genotypic strain distribution patterns that exist in the Mouse Genome Database for the C×B RI strains. The C×B RI strains have been typed for >160 simple sequence length polymorphisms that scan >80% of the mouse genome [25, 26]. Linkage analysis for candidate chromosomal regions was done by use of simple regression statistics [21] on a Map Manager computer program. Four candidate loci exhibited statistically significant linkage (P < .01). These loci are on chromosomes 3, 7, 8, and 18 and are linked to the strain distribution pattern with LOD (logarithm of the odds) scores of 1.6, 2.2, 1.8, and 2.1, respectively (figure 6).

The marker D3Mit86 is located on chromosome 3 at 56 centimorgans (cM) position. The marker D7Nds1 is located on chromosome 7 at 29 cM position. The markers D8Mit318 and D8Mit200 are located on chromosome 8 at 54 and 55 cM positions, respectively. These last two markers are 2 and 3 cM apart, respectively, from the gene encoding the macrophage migration inhibitory factor, pseudogene 5 (Mif-ps5). The marker D18Mit59 is located on chromosome 18 at 10 cM position [25]. This marker is 1 cM apart from the gene encoding the intercellular adhesion molecule-1 related sequence-1 (Icam-1 rs-1). Generations of backcross mice (BALB/c × C57BL/6) × C57BL/6 are currently being tested with these markers to confirm results in the C×B RI strains and locate with higher statistical significance the loci that control resistance to vaginal candidiasis after peripheral immunization.
Candidate chromosomal regions that control immune response to experimental vaginal candidiasis after peripheral immunization. Strain distribution pattern obtained from 12 phenotyped C×B recombinant inbred strains was compared with preexisting database for genetic markers on quantitative trait Map Manager computer program. Four candidate loci exhibited statistically significant linkage (P < .01) by simple regression statistics. Following markers exhibited statistical significant linkage: D7Nds1 (logarithm of odds [LOD] = 2.2), D18Mit59 (LOD = 2.1), D8Mit318 and D8Mit200 (LOD = 1.8), and D3Mit86 (LOD = 1.6). x axis represents microsatellite markers and their cM position on mouse chromosome; y axis represents LOD score.

BALB/c AnNCI, and BALB/c J were tested at the same time with the same experimental approach. The data suggest that in all substrains of BALB/c mice tested, subcutaneous immunization protects against a subsequent vaginal infection with C. albicans (data not shown). This contradictory result may therefore be the result of differences in the preparation of the cytoplasmic extract used. The CaX that was used for immunization in the present studies was prepared from supernatant containing cytoplasmic extract from disrupted yeasts, whereas the one used in the previous study was from culture supernatant concentrated on a molecular weight exclusion membrane. Therefore, our cytoplasmic extract may be more antigenically heterogeneous than that used in the previous study.

The exact mechanisms of how peripheral cell-mediated immunity is translated into mucosal immunity and protection is not clearly understood. It is apparent from the results presented in these studies that CD4 T cells play a role in protection against vaginal candidiasis after subcutaneous immunization. This result is consistent with a previous study in which CD4 lymphocytes were shown to play a significant role in resistance to mucosal candidiasis [14]. One mechanism by which CD4 lymphocytes may mediate resistance is by the production of cytokines, such as interferon-γ, which in turn can recruit effector cells, such as macrophages and neutrophils, that may protect against mucosal candidiasis [27, 28]. Humoral immunity may also be important in protection against C. albicans infection [29], and the role of this arm of the immune response needs to be further studied.

Two explanations for the observation that the ability of protection against a vaginal C. albicans infection after a subcutaneous immunization depends on the mouse strain tested can be envisaged. One explanation is that there is a unique set of T cells present in BALB/c but not in B6 mice that expresses a receptor for a ligand expressed on vaginal endothelial cells, enabling them to migrate to the vaginal mucosa on antigen recognition. It is unknown whether all T cells, once activated, have the ability to migrate to and protect the host against ‘‘non-self’’ in all organs of the body. Several reports have demonstrated strain-dependent differences in migration of various subsets of T cells [30].

A second possibility is that the difference in the ability of subcutaneous immunization to protect against subsequent vaginal infections resides in strain differences that enable memory T cells to migrate to the vaginal mucosa on infection. This genetic difference could regulate the presence of a tissue-specific homing receptor in the vaginal mucosa of BALB/c mice that is lacking in B6 mice. When BALB/c mice are vaginally infected, T cells that can respond to the antigens may receive the signal and migrate to the infected vaginal tissue and eliminate the pathogenic organism. Since this homing receptor may not be functionally expressed in B6 mice, the protective T cells are unable to respond to the vaginal infection.
B6 mice demonstrate a higher level of innate resistance to vaginal candidiasis than do BALB/c mice, which are more susceptible than B6 mice to the same intravaginal inoculum ($5 \times 10^6$ viable C. albicans) (figure 1). No studies have been done to examine the differences between these strains with respect to their ability to reduce vaginal candidiasis. Resistance or susceptibility to Leishmania major in B6 or BALB/c mice, respectively, has been associated with the ability of macrophages to synthesize nitric oxide following activation with interferon-γ [31]. Since production of nitric oxide by macrophages has been associated with resistance to murine vaginal candidiasis [28], it is possible that the difference in innate resistance to vaginal C. albicans between BALB/c and B6 mice is related to nitric oxide production. Additional studies are needed to provide a biologic basis for the genetic variation in innate resistance to C. albicans observed in inbred strains of mice.

RI mice are one of the most powerful tools in mammalian genetics for quick and efficient mapping of any genetic locus that differs between the two progenitor strains [26, 32]. The linkage analysis using 12 C×B RI strains revealed statistically significant correlations ($P < .01$) of strain distribution pattern with two markers on chromosome 8, located 2 and 3 cM apart, respectively, from the mif-ps5, and one marker on chromosome 18, located 1 cM apart from the Icam-1 rs1. In addition, other statistically significant associations of the strain distribution pattern with markers on other chromosomes were found ($P < .01$).

Linkage analysis revealed that the gene Icam-1 rs1, which is only 1 cM apart from the marker D18Mit59, is a strong candidate gene ($P < .01$) for controlling resistance to vaginal candidiasis after peripheral immunization. The Icam-1 rs1 gene was defined by linkage analysis with a DNA sequence probe from the intercellular adhesion molecule 1 (ICAM-1) [33]. Despite the high degree of homology between the sequences of the ICAM-1 and the Icam-1 rs1 genes, functionality of this latter gene is unknown. ICAM-1 has been shown to play a role in arresting circulating leukocytes and recruiting them to infected or otherwise inflamed tissue sites [34].

Evidence for genetic polymorphisms among cell adhesion molecules, such as ICAM-1, has been reported [35]. Genetic polymorphisms in endothelial cell adhesion molecules have been suggested to be important with regard to susceptibility to multifactorial disease processes that include an inflammatory component [35]. Moreover, studies have shown that other adhesion molecules, such as the mucosal addressin cell adhesion molecule 1 (MAdCAM-1), are uniquely expressed on endothelium in mucosal tissues [36]. Organ-specific differences in the ability of neutrophils to emigrate to different infected areas has been shown in other systems to be dependent on adhesion molecules such as ICAM-1 and P-selectin [37]. In addition, previous work done on ICAM-1 knockout mice has suggested that the loss of ICAM-1 significantly impairs host defense against disseminated candidiasis by impairing either neutrophil migration or phagocytic activation or both [38].

Genetic analysis also exhibited statistically significant linkage to Mif-ps5. The protein called macrophage migration inhibitory factor (MIF) is described to be a T lymphocyte product that inhibits the random migration of macrophages [39]. The Mif-ps5 gene contains mutations that give rise to truncated MIF-like proteins [40]. However, the possibility of a functional Mif pseudogene in the mouse genome cannot be excluded [40]. It appears possible that in strains such as B6, which are not protected against a vaginal challenge after peripheral immunization, the Mif-ps5 pseudogene could be up-regulated, resulting in impairment of macrophage ability to migrate to the site of infection.

Previous studies have demonstrated a genetic control of resistance and susceptibility in mice to systemic infections with C. albicans on intravenous inoculation [13, 41-43]. Additionally, mouse strains congenic with respect to the mouse major histocompatibility complex, H-2, have been shown to exhibit different kinetics of responses to oral mucosal infection with C. albicans and the resolution of the infection [44]. These studies also demonstrated that the direct topical application of C. albicans results in the generation of different types of immune responses between BALB/c and DBA/2 mouse strains, since delayed-type hypersensitivity reactivity could be demonstrated in DBA/2 but not BALB/c mice. These studies, therefore, support the possibility that genetic differences among mouse strains may regulate the observed differences in effectiveness of protection against vaginal challenge conferred by subcutaneous immunization.

In summary, this study demonstrates that it is possible to achieve significant protection against vaginal infection with C. albicans by subcutaneous immunization in BALB/c mice but not in B6 mice. Moreover, protection conferred by immunization in BALB/c mice seems to be mediated by a secondary immune response associated with a CD4 population of T cells. Our data demonstrate that the ability of BALB/c mice to generate protective immune responses to vaginal challenge with C. albicans after subcutaneous immunization is genetically determined. The difference in the ability to protect different strains of mice from vaginal C. albicans infection may, at least in part, offer an explanation for the differences in susceptibility of women to vaginal infections. Furthermore, gene mapping studies in the mouse system may provide valuable tools for elucidating gene loci regulating vaginal immunity.

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**References**