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# Resistance of T-Cell Receptor δ-Chain-Deficient Mice to Experimental *Candida albicans* Vaginitis

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**Conditions consistent with tolerance or immunoregulation have been observed in experimental *Candida albicans* vaginal infections. The present study investigated the role of γ/δ T cells in experimental vaginal candidiasis. Results showed that T-cell receptor δ-chain-knockout mice had significantly less vaginal fungal burden when compared to wild-type mice, suggesting an immunoregulatory role for γ/δ T cells in *Candida* vaginitis.**

Recurrent vulvovaginal candidiasis, caused predominantly by *Candida albicans*, is an opportunistic fungal infection affecting an estimated 5 to 10% of otherwise healthy women of childbearing age (20). Cell-mediated immunity (CMI) involving Th1-type α/β T-cell-receptor-positive ($TCR^+$) $CD4^+$ T cells is the predominant host defense mechanism against *C. albicans* at mucosal surfaces (1, 18). However, studies of women with recurrent vulvovaginal candidiasis and an experimental murine model of vaginal candidiasis show little if any protective role for systemic and mucosal CMI (3, 4, 6, 7). Together, these results suggested that some form of tolerance or immunoregulation inhibits more profound CMI against vaginal candidiasis.

We and others have shown that vagina-associated T cells are phenotypically distinct from those in the periphery, with high percentages of γ/δ $TCR^+$ T cells compared to those in the periphery (8, 10, 11, 16). The purpose of the current study was to more clearly define the role of γ/δ $TCR^+$ T cells in immunity to experimental vaginal candidiasis using TCR $\delta^{-/-}$ mice that are homozygous for the $Tcrd^{tm/MomC}$ mutation and consequently deficient in γ/δ T cells in all adult lymphoid and epithelial organs (12).

To assess the role of γ/δ $TCR^+$ T cells in the host response to experimental vaginal candidiasis, female TCR $\delta^{-/-}$ mice (C57BL/6 background) (9) and wild-type mice (Jackson Laboratory, Bar Harbor, Maine), 8 to 12 weeks of age, were vaginally inoculated with stationary-phase *C. albicans* 3153A blastoconidia as previously described (5). Quantitative culture of vaginal lavage fluid demonstrated that the vaginal fungal burden in TCR $\delta^{-/-}$ mice was significantly less than in wild-type mice (Fig. 1) ($P < 0.05$ and 0.001 on days 4 and 10, respectively). This suggested that the presence of γ/δ $TCR^+$ T cells increased the susceptibility of mice to experimental *C. albicans* vaginitis. To identify a systemic immune correlate for the resistance of TCR $\delta^{-/-}$ mice to experimental *C. albicans* vaginitis, *Candida*-specific delayed-type hypersensitivity (DTH) was measured as previously reported (5). Results showed that DTH values for TCR $\delta^{-/-}$ and wild-type mice were similarly positive on day 10 postinoculation (0.312 ± 0.09 mm and 0.25 ± 0.06 mm, respectively) and negative on day 4, consistent with the development of *Candida*-specific DTH following infection (5). Thus, γ/δ $TCR^+$ T cells have no demonstrable influence on *Candida*-specific DTH and DTH could not explain the observed infection results.

In studies examining local cellular changes during infection, flow cytometric analysis performed as previously described (8) showed no significant changes in the percentages of vaginal $CD4^+$ or $CD8^+$ α/β $TCR^+$ cells in TCR $\delta^{-/-}$ mice compared to those in wild-type mice on days 4 and 10 postinoculation. As a confirmation, vaginal tissue sections prepared from TCR $\delta^{-/-}$ and wild-type mice on day 10 postinoculation and stained with hematoxylin and eosin (H&E) (Hema-3 staining kit; Fisher Scientific, Pittsburgh, Pa.) showed no evidence of a leukocyte infiltrate or any changes in the local cellular composition as a result of infection (Fig. 2).

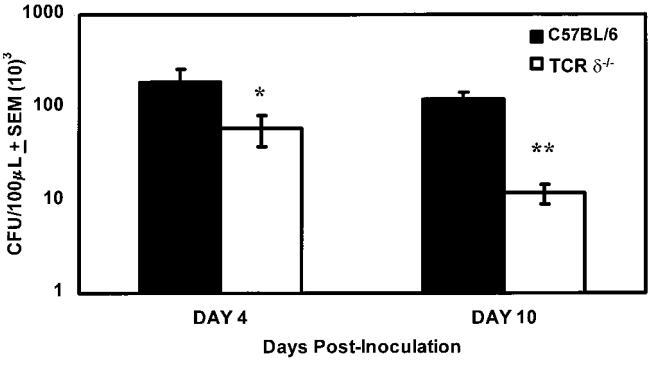


FIG. 1. Experimental vaginal candidiasis in the absence of γ/δ $TCR^+$ T cells. Groups of 10 estrogen-treated TCR $\delta^{-/-}$ and wild-type C57BL/6 mice were inoculated vaginally with $5 \times 10^4$ *C. albicans* blastoconidia. Mice were sacrificed on days 4 and 10 postinoculation, and vaginal fungal burden was quantified by culture of vaginal lavage fluid. Data are mean numbers of CFU ($10^3$) ± standard errors of the means (SEM) for four experiments. *, $P < 0.05$; **, $P < 0.0001$.

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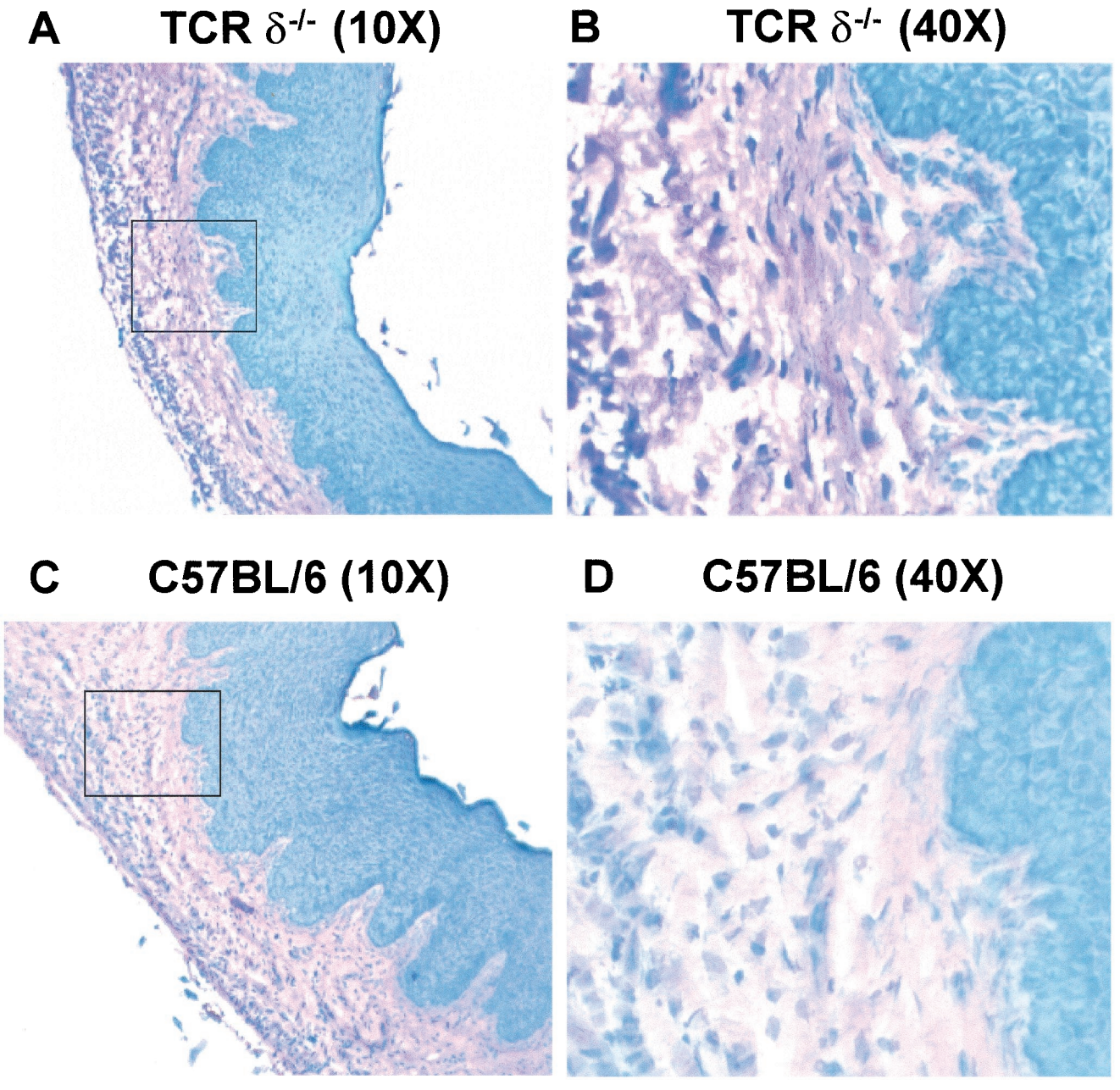


FIG. 2. H&E staining of vaginal tissue during experimental vaginal candidiasis in the absence of γ/δ TCR$^+$ T cells. Whole vaginal tissues from estrogen-treated TCR $\delta^{-/-}$ and wild-type C57BL/6 mice were excised on day 10 postinoculation, frozen in optimal-cutting-temperature medium, and sectioned (10 μm). Sections were stained with H&E using a Hema-3 staining kit and examined at ×10 (A and C) and x40 (B and D) magnification. The ×40 image is taken from the boxed region of the ×10 image.

Local immunity was evaluated by the presence of Th1-type (gamma interferon and interleukin-12 [IL-12]) and Th2-type (IL-4, IL-10, and transforming growth factor $\beta_1$) cytokines in infected TCR $\delta^{-/-}$ and wild-type mice by enzyme-linked immunosorbent assay (BD Pharmingen or Genzyme Diagnostics, Cambridge, Mass) with concentrations normalized to total protein (BCA kit; Pierce, Rockford, Ill.) as previously described (22). Although cytokines were detected throughout infection as per previous studies (22), including high concentrations of transforming growth factor $\beta_1$ suggestive of local immunoregulation, no differences in vaginal cytokine concentrations were observed between groups on days 4 and 10 postinoculation to explain the increased resistance of TCR $\delta^{-/-}$ mice to infection (data not shown).

As local CMI did not reveal any correlates for the increased resistance of TCR $\delta^{-/-}$ mice to *C. albicans* vaginal infection, we next examined *Candida*-specific antibodies in vaginal lavages (pooled from 8 to12 animals per group) collected from TCR $\delta^{-/-}$ and wild-type mice by enzyme-linked immunosorbent assay using *Candida* soluble cytoplasmic substances as the capture antigen as described elsewhere (L. Cárdenas-Freytag, C. Steele, F. L. Wormley, Jr., E. Cheng, J. D. Clements, and

P. L. Fidel, Jr., submitted for publication). On days 4 and 10 postinoculation negligible levels of vaginal *Candida*-specific immunoglobulin G (IgG) and IgA were detected in both groups of mice (data not shown). Interestingly, a similar lack of *Candida*-specific IgG and IgA was observed in CBA/J mice both during a primary vaginal infection (unpublished results) and following mucosal immunization with *Candida* antigen and the mucosal adjuvant LT(R192G) (Cárdenas-Freytag et al., submitted).

We also examined mechanisms of innate resistance. Vaginal epithelial cells from uninfected mice have been shown to inhibit the growth of *C. albicans* in vitro (21). Vaginal epithelial cells from TCR $\delta^{-/-}$ and wild-type mice were similarly capable of inhibiting the growth of *C. albicans* 50 to 65% at an effector-to-target (epithelial cell to *C. albicans*) ratio of 80:1 (data not shown), consistent with previous results obtained with CBA/J mice (21). We also investigated the presence of $NO_2$ in vaginal tissue homogenates of infected mice using the Griess technique (19), based on the nitric oxide-dependent candidicidal activity of macrophages (13). Results showed that $NO_2$ levels in vaginal homogenates were similar between TCR $\delta^{-/-}$ and wild-type mice on days 4 ($18.2 \pm 1.4$ and $23.5 \pm 2.2$ μmol/mg of protein in TCR $\delta^{-/-}$ and wild-type mice, respectively) and 10 ($29.7 \pm 3.4$ and $23.6 \pm 2.1$ μmol/mg of protein in TCR $\delta^{-/-}$ and wild-type mice, respectively) postinoculation. Thus, the enhanced resistance to infection in the absence of γ/δ $TCR^+$ T cells did not appear to be associated with vaginal epithelial cell-mediated anti-*Candida* activity or $NO_2$ production.

The results of this study suggesting an immunoregulatory role for γ/δ $TCR^+$ T cells leading to exacerbation of infections is supported by other models of experimental *Salmonella enterica* serovar Choleraesuis (2) and *Listeria monocytogenes* (17) infection. Although resistance to lethal infection with *Salmonella enterica* serovar Choleraesuis was associated with a reduced inflammatory response, like in the present study, the mechanism for resistance to *L. monocytogenes* is yet to be elucidated. In contrast, the findings herein are contrary to the increased susceptibility to infection observed in other experimental models (14, 15) and to the increased susceptibility to experimental vaginal *Candida* infection in mice depleted of γ/δ $TCR^+$ T cells observed in a study using complement-fixing antibodies (13). In the latter study, however, depletion of vaginal γ/δ $TCR^+$ T cells was not confirmed. Although the mechanism by which γ/δ $TCR^+$ T cells increase susceptibility to infection is unknown, the resistance observed was consistent and reproducible. Thus, it appears that γ/δ $TCR^+$ T cells are involved in some form of tolerance or immunoregulation inhibiting a more profound response to *C. albicans* at the vaginal mucosa. Additionally, this immunoregulatory role appears to be at both innate and adaptive levels since the effects were seen at days 4 and 10 postinoculation. An immunoregulatory role would be important to the survival of this vaginal commensal organism and benefit the host as well by the limited inflammatory response upon exposure to *Candida*. While γ/δ $TCR^+$ T cells are not abundant at the vaginal mucosa, their high percentage of total cells compared to that in the periphery (8), as well as their unique TCR variable gene segments ($V\gamma_4V\delta1$) (11), may be important to their site-specific immunoregulatory role.

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## REFERENCES


1. **Cenci, E., A. Mencacci, R. Spaccapelo, L. Tonnetti, P. Mosci, K. H. Enssle, P. Puccetti, L. Romani, and F. Bistoni.** 1995. T helper cell type 1 (Th1)- and Th2-like responses are present in mice with gastric candidiasis but protective immunity is associated with Th1 development. J. Infect. Dis. **171:**1279–1288.
2. **Emoto, M., H. Nishimura, T. Sakai, K. Hiromatsu, H. Gomi, S. Itohara, and Y. Yoshikai.** 1995. Mice deficient in γδ T cells are resistant to lethal infection with *Salmonella choleraesuis*. Infect. Immun. **63:**3736–3738.
3. **Fidel, P. L., Jr., J. L. Cutright, and J. D. Sobel.** 1995. Effects of systemic cell-mediated immunity on vaginal candidiasis in mice resistant and susceptible to *Candida albicans* infections. Infect. Immun. **63:**4191–4194.
4. **Fidel, P. L., Jr., M. E. Lynch, D. H. Conaway, L. Tait, and J. D. Sobel.** 1995. Mice immunized by primary vaginal *C. albicans* infection develop acquired vaginal mucosal immunity. Infect. Immun. **63:**547–553.
5. **Fidel, P. L., Jr., M. E. Lynch, and J. D. Sobel.** 1993. *Candida*-specific cell-mediated immunity is demonstrable in mice with experimental vaginal candidiasis. Infect. Immun. **61:**1990–1995.
6. **Fidel, P. L., Jr., M. E. Lynch, and J. D. Sobel.** 1994. Effects of preinduced *Candida*-specific systemic cell-mediated immunity on experimental vaginal candidiasis. Infect. Immun. **62:**1032–1038.
7. **Fidel, P. L., Jr., M. E. Lynch, and J. D. Sobel.** 1995. Circulating CD4 and CD8 T cells have little impact on host defense against experimental vaginal candidiasis. Infect. Immun. **63:**2403–2408.
8. **Fidel, P. L., Jr., N. A. Wolf, and M. A. KuKuruga.** 1996. T lymphocytes in the murine vaginal mucosa are phenotypically distinct from those in the periphery. Infect. Immun. **64:**3793–3799.
9. **Fujihashi, K., J. R. McGhee, M. N. Kweon, M. D. Cooper, S. Tonegawa, I. Takahashi, T. Hiroi, J. Mestecky, and H. Kiyono.** 1996. gamma/delta T cell-deficient mice have impaired mucosal immunoglobulin A responses. J. Exp. Med **183:**1929–1935.
10. **Ibraghimov, A. R., R. E. Sacco, M. Sandor, Z. Iakoubov, and R. G. Lynch.** 1995. Resident $CD4^+$ αβ T cells of the murine female genital tract: a phenotypically distinct T cell lineage that rapidly proliferates in response to systemic T cell activation stimuli. Int. Immunol. **7:**1763–1769.
11. **Itohara, S., A. G. Farr, J. J. Lafaille, M. Bonneville, Y. Takagaki, W. Haas, and S. Tonegawa.** 1990. Homing of a gamma/delta thymocyte subset with homogenous T-cell receptors to mucosal epithelia. Nature **343:**754–757.
12. **Itohara, S., P. Mombaerts, J. Lafaille, J. Iacomini, A. Nelson, A. R. Clarke, M. L. Hooper, A. Farr, and S. Tonegawa.** 1993. T cell receptor delta gene mutant mice: independent generation of alpha beta T cells and programmed rearrangements of gamma delta TCR genes. Cell **72:**337–348.
13. **Jones-Carson, J., A. Vazquez-Torres, H. C. van der Heyde, T. Warner, R. D. Wagner, and E. Balish.** 1995. γ/δ T cell-induced nitric oxide production enhances resistance to mucosal candidiasis. Nat. Med. **1:**552–557.
14. **Mombaerts, P., J. Arnoldi, F. Russ, S. Tonegawa, and S. H. Kaufmann.** 1993. Different roles of alpha beta and gamma delta T cells in immunity against an intracellular bacterial pathogen. Nature **365:**53–56.
15. **Moore, T. A., B. B. Moore, M. W. Newstead, and T. J. Standiford.** 2000. Gamma delta-T cells are critical for survival and early proinflammatory cytokine gene expression during murine Klebsiella pneumonia. J. Immunol. **165:**2643–2650.
16. **Nandi, D., and J. P. Allison.** 1991. Phenotypic analysis and gamma/delta-T cell receptor repertoire of murine T cells associated with the vaginal epithelium. J. Immunol. **147:**1773–1778.
17. **O'Brien, R. L., X. Yin, S. A. Huber, K. Ikuta, and W. K. Born.** 2000. Depletion of a gammadelta T cell subset can increase host resistance to a bacterial infection. J. Immunol. **165:**6472–6479.
18. **Odds, F. C.** 1988. Candida and candidosis, p. 104–110. University Park Press, Baltimore, Md.
19. **Schwacha, M. G., J. J. Meissler, Jr., and T. K. Eisenstein.** 1998. *Salmonella typhimurium* infection in mice induces nitric oxide-mediated immunosuppression through a natural killer cell-dependent pathway. Infect. Immun **66:**5862–5866.
20. **Sobel, J. D.** 1992. Pathogenesis and treatment of recurrent vulvovaginal candidiasis. Clin. Infect. Dis. **14:**S148–S153.
21. **Steele, C., H. Ozenci, W. Luo, M. Scott, and P. L. Fidel, Jr.** 1999. Growth inhibition of *Candida albicans* by vaginal cells from naive mice. J. Med. Mycol. **37:**251–260.
22. **Taylor, B. N., M. Saavedra, and P. L. Fidel, Jr.** 2000. Local Th1/Th2 cytokine production during experimental vaginal candidiasis. J. Med. Mycol. **38:**419–431.


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