INFECTION AND IMMUNITY, Aug. 2004, p. 4878–4880 Vol. 72, No. 8
0019-9567/04/$08.00+0 DOI: 10.1128/IAI.72.8.4878–4880.2004

Genetic Susceptibility of Mice to *Candida albicans* Vaginitis Correlates with Host Estrogen Sensitivity

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Received 16 January 2004/Returned for modification 1 March 2004/Accepted 3 May 2004

We compared susceptibility to *Candida* vaginitis in derived murine substrains differing in sensitivity to estrogen (CD-1 and CD10, resistant; CD3 and C57BL/6 responsive), and in F1 crosses. The order of decreasing resistance was CD-1 ≥ CD10 ≥ CD10 × CD3F1 > CD10 × B6F1 > CD3 > C57BL/6 and correlated with estrogen responsiveness in endocrine disruptor assays. Resistance to *Candida* vaginitis appears additive in CD10 × B6F1 animals and dominant in CD10 × CD3F1 animals.

Vaginal *Candida* infection is associated with risk factors, including estrogenization status (1). The incidence of vaginal candidiasis increases during pregnancy, during the luteal phase of the menstrual cycle when estrogen and progesterone levels are elevated, with estrogenic contraceptive use, and with estrogen replacement therapy (6). Estrogen levels seem to be the main hormonal factor affecting host susceptibility (7). In addition, *Candida albicans* produces an estrogen-binding protein (8, 9, 12, 16, 20), and mammalian estrogens and phytoestrogens may increase its pathogenicity (10, 18, 19). Thus, defining the host-fungal interplay during candidal vaginitis is important to our understanding of this disease.

In previous studies (4, 17), our investigators noted that several inbred and outbred strains of mice were susceptible to vaginal infection, whereas only a single outbred strain of mice, CD-1, was resistant (4). Differential susceptibility to infection in mouse strains has been noted for a number of fungal infections and provides a tool for the study of mechanisms of host resistance.

The establishment of vaginal candidiasis in mice is dependent on prolonged pseudoestrus, usually induced with exogenous 17-β-estradiol (E_2). Although most strains of mice show a consistent response to exogenous E_2, CD-1 mice are much less responsive (14, 15). In most strains, pubertal exposure to as little as 0.25 μg of E_2 per g of body weight causes significant reduction of testes weight, spermatogenesis, and spermatid development (14, 15). In contrast, CD-1 mice are >16-fold less responsive to the effects of E_2 on these parameters (14, 15), possibly due to high levels of testicular sulfotransferase (15).

A program of more than 20 generations of full sibling matings from CD-1 strain mice has developed inbred substrains that differ in their response to E_2 (J. L. Spearow, D. R. Morris, U. Wong, R. Altafi, T. T. Stearns, K. J. Mogel, M. R. Sanford, S. W. Eteiwi, and M. Barkley, Annu. Meet. Endocr. Soc., abstr. P3-74, p. 511, 2002). The testes weight response to E_2 showed that some substrains were only slightly more sensitive than CD-1 and others were much more sensitive. However, all CD-1-derived inbred strains were significantly less responsive to E_2 than were C57BL/6J strains (Spearow et al., Annu. Meet. Endocr. Soc., 2002; J. L. Spearow, unpublished data). We have used these substrains to study the possible role of relative estrogen responsiveness in experimental *Candida* vaginitis infection, whether resistance to infection correlated with estrogen responsiveness, and whether CD-1-derived inbred strains could be used to define genetic differences in susceptibility to *Candida* vaginitis.

(This research was presented in part at the 15th Congress of the International Society for Human and Animal Mycology, San Antonio, Tex., May 2003 [R. Parmar, J. L. Spearow, M. Espiritu, K. V. Clemons, and D. A. Stevens, 15th Congr. Int. Soc. Human Animal Mycol., abstr. 274, 2003].)

Model. A murine model of vaginitis due to *C. albicans* (strain 5) was performed as described previously (4, 13, 17). Pseudoestrus was induced with 0.5 mg of estradiol valerate in sterile sesame oil (4, 17). All CD-1 and associated substrains, C57BL/6 (B6), and F1 hybrid mice (Table 1) were bred in the facilities of the University of California at Davis. In the initial experiment, animals were 11 to 12 weeks old, and their weight ranged from 22 to 36 g. In the second experiment, all the animals were 6 to 7 weeks old (weight range, 18 to 29 g). Animals were infected by vaginal lavage with 20 μl of a suspension containing 3×10^8 yeast/ml. Vaginal swabs were taken on days 1 and 6 postinfection to determine CFU by quantitative plating (4, 17). These studies were done with institutional approval from the California Institute for Medical Research and the University of California at Davis.

All animal experimentation was done following the guidelines of the U.S. Department of Agriculture and the National Institutes of Health with the approval of the institutional animal care and use committees of the California Institute for Medical Research and The University of California at Davis.

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TABLE 1. Mouse strains, estrogen responsiveness, and susceptibility to *Candida* vaginitis

Expt and mouse strain	Susceptibility to disruption of testes function by estrogen[a]	Susceptibility to estrogen-induced *Candida* vaginitis[b]
Expt 1		
CD-1		Resistant
CD10		Resistant
CD3		Susceptible
CD10 × CD3F1		Intermediate
C57BL/6J		Most susceptible
Expt 2		
CD-1	Resistant	Resistant
CD10	Resistant	Resistant
CD3	Responsive	Susceptible
CD10 × CD3F1	Resistant	Resistant
CD10 × C57BL/6JF1	Intermediate	Intermediate
C57BL/6J	Most responsive	Most susceptible

[a] Susceptibility based on dose-response inhibition of spermatid production and testes weight by estradiol in pubertal mice, as determined in other studies and reported previously (14). "Responsive" indicates that <40 μg of E_2 per g of body weight caused a ≥50% inhibition of testes function; "resistant" indicates <50% inhibition of testes function at the same dose. "Intermediate" indicates a response between those of the parental strains.

[b] "Susceptible" was defined as no change or an increase in CFU between days 1 and 6; "resistant" was defined as a significant decrease in CFU between days 1 and 6. "Intermediate" indicates the mean CFU recovered at day 6 ranked between those recovered from the parental strains.

Statistics. Log_{10} CFU-transformed data were analyzed by an analysis of variance (ANOVA) using Fisher's protected least significant difference test for multiple range comparisons. Comparison within a strain for day 1 versus day 6 samples was done using a Mann-Whitney U test.

Experiment 1. In the initial experiment, we examined the relative susceptibility of various mouse strains and substrains (Table 1) to vaginal infection with *C. albicans* and its correlation with estrogen sensitivity. The influence of the variables of strain and day of infection on CFU were significant by ANOVA ($P < 0.0001$). The results showed that estrogen-primed positive control C57BL/6J (B6) strain mice were susceptible to *Candida* vaginitis, as defined by either an increase or no change in CFU from day 1 postinfection to day 6 postinfection (Table 2). These animals carried significantly higher mean CFU than other strains of mice tested on both days 1 and 6 (Table 2). In contrast, CD-1 mice were resistant, defined as having a significant decrease in CFU from day 1 to 6, with CFU significantly decreasing by >100-fold. The E_2-resistant CD10 mice also had a significant decrease in CFU, whereas E_2-responsive CD3 mice had a nonsignificant decrease in mean CFU, unlike CD-1 or CD10, but were not as susceptible as B6 mice (Table 2). On day 6, CD3 mice had significantly higher mean CFU burdens than CD10 or CD-1 by Fisher's LSD test ($P < 0.05$).

CD10 × CD3F1 hybrid mice showed intermediate results, with the CFU recovered falling between those recovered from their parental strains. However, there were no statistical differences in burdens on day 1 or 6 after infection among the CD-1, CD3, CD10, or CD10 × CD3F1 strain mice. In addition, the CD10 × CD3F1 mice were not significantly different from the CD10 × CD3 midparental average.

Experiment 2. In this experiment, we sought to confirm the results of the initial study, as well as to examine younger animals, to determine whether age was a factor in addition to E_2 responsiveness. The effects of strain, day, and strain-day interaction on comparative CFU recovered were all significant by ANOVA ($P < 0.01$). Only B6 mice showed an increase in CFU from day 1 to day 6, whereas all other strains showed a decrease in CFU (Table 2). B6 mice carried significantly higher burdens of yeast on day 6 than all other strains of mice ($P < 0.05$) (Table 2). Estrogen-responsive CD3 mice had significantly higher burdens on day 6 than did CD10 × CD3F1 mice ($P < 0.05$). CD3 mice carried >20-fold higher mean CFU on day 6 than did CD-1 or CD10 mice ($P < 0.05$) (Table 2).

The CD10 × B6F1 hybrid mice showed CFU burdens intermediate to those of the parental resistant (CD10) and susceptible (B6) strains (Table 2). CFU from CD10 × B6F1 mice did not differ significantly from the midparental average of log_{10} 2.78, with a nonsignificant dominance deviation of −18.5%. Thus, resistance to vaginitis appears mainly to be additive in the CD10 × B6F1 cross.

TABLE 2. Recovery of *C. albicans* from the vaginas of infected mice

Expt and mouse strain	No. of mice	Log_{10} geometric mean CFU (95% CI)[d] [no. free of infection]		Fold change in CFU from day 1 to day 6	*P* value, day 1 vs day 6[a]	Strain differences on day[b]
		Day 1	Day 6			
Expt 1						
CD-1	11	2.83 (1.8–3.8)	0.66 (0–1.3) [7]	138, decrease	0.003	A
CD10	14	2.22 (1.5–2.9)	0.54 (0.09–0.99) [9]	46, decrease	0.002	A
CD3	12	2.87 (2.0–3.7)	1.85 (0.77–3.3) [5]	8, decrease	NS[c]	B
CD10 × CD3F1	8	2.83 (1.8–3.9)	1.33 (0–2.9) [5]	32, decrease	NS	AB
C57BL/6	10	4.00 (3.6–4.4)	3.97 (3.7–4.2) [0]	No change	NS	C
Expt 2						
CD-1	15	3.32 (2.73–3.91)	1.46 (0.82–2.09) [4]	73, decrease	0.0004	AB
CD10	15	3.36 (2.90–3.81)	1.48 (0.39–2.57) [9]	75, decrease	0.056	AB
CD3	14	3.57 (3.04–4.10)	2.85 (1.94–3.76) [2]	5.3, decrease	NS	C
CD10 × B6F1	15	3.64 (3.24–4.03)	2.27 (1.50–3.04) [2]	23, decrease	0.008	BC
CD10 × CD3F1	13	2.35 (1.37–3.33)	0.58 (0–1.18) [9]	59, decrease	0.006	A
C57BL/6	14	3.92 (3.66–4.18)	4.09 (3.67–4.50) [0]	1.5, increase	NS	D

[a] *P* values were determined with the Mann-Whitney U test.

[b] Comparisons of CFU with various strains at day 6 were done by using Fisher's least significant difference multiple range test. Strains with the same letter designation were not significantly different ($P > 0.05$), while strains with different letter designations were significantly different ($P < 0.05$).

[c] NS, not significant.

[d] CI, confidence interval.

Unlike the results of the initial study, the CD10 × CD3F1 mice were not intermediate to their parental strains and had significantly lower CFU than the CD10 × CD3 midparental average ($\log_{10}$ 2.16 CFU), as well as the CD3 parent ($P < 0.05$). The CD10 × CD3F1 mice showed a dominance deviation of −73% from the midparental average ($P < 0.001$). The CD10 × CD3F1 animals also carried the lowest mean burdens on both days of sampling. These data indicate dominance or other nonadditive gene action controlling this trait in the CD10 × CD3F1 mice, i.e., resistance is dominant. Also note that the CD10 × B6F1 mice carried significantly higher burdens on day 6 than did the CD10 × CD3F1 mice ($P < 0.05$) (Table 2). Overall, these data are suggestive that susceptibility in this model is controlled by additive and nonadditive gene action.

The inbreeding from CD-1 has resulted in a divergence in susceptibility to E_2-induced *Candida* vaginitis between CD10 and CD3 strain mice. Although CD-1 mice are very resistant to E_2 endocrine disruption, our data suggest that this outbred line is segregating at loci controlling resistance to vaginitis. This is supported by the finding of an occasional CD-1 mouse with only moderate resistance to endocrine disruption by E_2 (14; Spearow, unpublished). Thus, genetically homogenous inbred CD10 strain mice are more likely to provide a reproducible and genetically defined animal model for resistance to E_2-induced *Candida* vaginitis than are outbred CD-1 mice. Comparisons of resistant CD10 strain mice and susceptible B6 strain mice provide divergent models for genetically mapping and determining the mechanism of action of genes controlling susceptibility to E_2-induced *Candida* vaginitis. Comparisons of CD10 and CD3 inbred strain mice also provide genetically defined models for identifying the genes still segregating in CD-1 that control susceptibility to E_2-induced *Candida* vaginitis.

Although it did not appear to influence the overall results, the older animals (i.e., 11 to 12 weeks) had lower mean CFU than did the younger animals (6 to 7 weeks). The difference in age likely explains the modest differences between experiments, as even smaller differences in age (e.g., 2 weeks) result in profound differences in resistance to various fungal infections (e.g., blastomycosis [2] and other mycoses [3, 5, 11]). The age range within each experiment did not appear to influence the results (based on analysis of scattergram results of each experiment) (data not shown). Additional studies are needed to clarify this aspect.

These data corroborate our previous study (4) showing that CD-1 mice are much less susceptible to *Candida* vaginitis, and the data provide further evidence that susceptibility to *Candida* vaginitis correlates well with strain responsiveness to estrogen disruption of reproductive development and spermatogenesis (14, 15; Spearow et al., Annu. Meet. Endocr. Soc., 2002). There were significant differences in susceptibility to vaginitis among the four mouse strains and two F1 crosses, and the rankings from most resistant to most susceptible were CD-1 ≥ CD10 ≥ CD10 × CD3F1 > CD10 × B6F1 > CD3 > C57BL/6. Furthermore, this order correlated with responsiveness to E_2 in endocrine disruptor assays. Both F1 hybrids in the second experiment were more resistant to infection than were their susceptible parent (i.e., CD3 or B6), which clearly indicates that resistance is not a recessive trait. Thus, resistance to vaginal infection appears to be additive in the CD10 × B6F1 cross and dominant in the CD10 × CD3F1 cross. However, additional studies are required to determine the number of segregating loci, to map, and to identify the genes involved. These inbred strains of mice provide a suitable and valuable genetically defined model for mapping, characterizing, and identifying genes controlling or responsible for E_2-induced susceptibility to *Candida* vaginitis.

We thank Laurence F. Mirels for helpful suggestions.

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Editor: T. R. Kozel