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Increased Susceptibility of Secretor Factor Gene *Fut2*-Null Mice to Experimental Vaginal Candidiasis

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Fut2-LacZ-null mice, which are a model of the human ABO and Lewis nonsecretor group, display increased susceptibility to experimental yeast vaginitis, indicating a role for α(1,2)fucosylated cervical glycans in mucosal defense. However, the lack of significant effect of competitive inhibition by exogenous neoglycoproteins in this study emphasizes the complexity of *Candida*-epithelial cell adhesion events.

Recurrent vulvovaginal candidiasis (RVVC) is a mucosal infection caused by the opportunistic fungus *Candida albicans* which affects up to 5 to 10% of women of reproductive age (22, 23). The immune response elicited during an episode of RVVC differs from the classical host defense mechanism, as infection occurs despite normal *Candida*-specific Th1-type cell-mediated immunity (9, 16). Protection from RVVC is believed to be acquired locally, possibly involving incompletely defined vaginal epithelial carbohydrate adhesion molecules (2, 24).

A common null mutation within the coding region of the α(1,2)fucosyltransferase gene, *FUT2* (secretor factor gene), leads to ABO and Lewis histo-blood group antigen nonsecretion from mucosal tissues in approximately 20% of humans, with ethnic variation (13, 14). Nonsecretor status has been associated with differences in susceptibility to several infections, including infections with Norwalk virus (15), human immunodeficiency virus (1), *Escherichia coli* (17, 21), *Staphylococcus aureus* (20), *Campylobacter jejuni* (19), calicivirus (18), and *C. albicans* (5). While the mechanism of how *C. albicans* interacts with nonsecretors is not known, a host-microbe adhesion mechanism is supported by in vitro studies that have demonstrated binding of various fucosylated oligosaccharides to germ tubes of *C. albicans* (3, 4, 6, 25). We report here the development of a mouse model for the nonsecretor phenotype to test the importance of α(1,2)fucosylated glycans in vivo during experimental vaginal candidiasis.

Absence of endocervical and vaginal α(1,2)fucosylated glycans in Fut2-LacZ-null mice. The expression pattern of *Fut2* and resulting α(1,2)fucosylated glycans in the female lower reproductive tract was examined in 8- to 10-week-old female mutant mice (Fut2-LacZ-null mice) that contained a targeted replacement of the *Fut2* open reading frame with a bacterial *lacZ* reporter gene (8). The original mutant mice were backcrossed 10 generations to C57BL/6J mice (Jackson Laboratory, Bar Harbor, Maine), and the resulting congenic strain used for this study was designated B6.129X1-*Fut2*^{tm1Sdo} (MGI accession ID no. 2183220). A second line of mutant mice lacking the coding region of another α(1,2)fucosyltransferase gene, *Fut1*, was similarly backcrossed for 10 generations to C57BL/6J mice and was designated B6.129X1-*Fut1*^{tm1Sdo} (MGI accession ID no. 2183219) and used as genetic background controls.

Using a 5-bromo-4-chloro-3-indolylβ-D-glucuronic acid (X-Gal) staining method (7), specific nuclear LacZ staining was detected in the glandular and lumenal epithelium of the endocervix of Fut2-LacZ-null mice in estrus (Fig. 1A), but not in wild-type mice (Fig. 1B). Lectin staining with *Ulex europaeus* agglutinin I (UEA-I) showed loss of α(1,2)fucosylated glycans from the endocervix and upper vagina of Fut2-LacZ-null mice (Fig. 1C), while lectin staining was preserved at the apical surface of the epithelium of the endocervix and upper vagina in wild-type mice (Fig. 1D), indicating that *Fut2* is essential for the production of α(1,2)fucosylated glycans within the endocervix and vagina. Given epidemiological evidence that nonsecretors are more susceptible to RVVC (5) and the implication of fucose involvement in *C. albicans* adhesion from various in vitro studies (4, 25), we postulated that α(1,2)fucosylated glycans carried on secreted endocervical mucins protect the vaginal epithelium by sequestering and thereby preventing *C. albicans* from adhering to vaginal epithelial cells. This potential defense mechanism would be reduced in nonsecretor women. The estrogen-regulated expression of FUT2 may thus represent innate protection against monthly infection with *C. albicans* by increasing α(1,2)fucosylated glycan secretion at times most risky for colonization associated with elevated estrogen.

Fut2-LacZ-null mice display increased susceptibility to *C. albicans* during experimental vaginal candidiasis compared to wild-type and Fut1-null control mice. To test for and quantify potential differences between wild-type and mutant mice during experimental vaginal candidiasis, female C57BL/6J wild-type, Fut1-null, and Fut2-LacZ-null mice were maintained in pseudoestrus by using subcutaneous 0.5-mg, 21-day controlled release 17β-estradiol pellets (Innovative Research of America, Sarasota, Fla.). Following intravaginal inoculation with 10 μl containing 5×10^5 stationary-phase *C. albicans* (3153A) cells (10, 11) mice were euthanized at 4, 7, or 14 days, and the vagina and cervix were removed en bloc, homogenized in phosphate-buffered saline (12), and plated on Sabouraud dextrose agar plates to determine CFU counts (CFU per organ). This study was approved for humane animal use. Hematoxylin and eosin staining of paraffin-embedded vaginas of infected mice at

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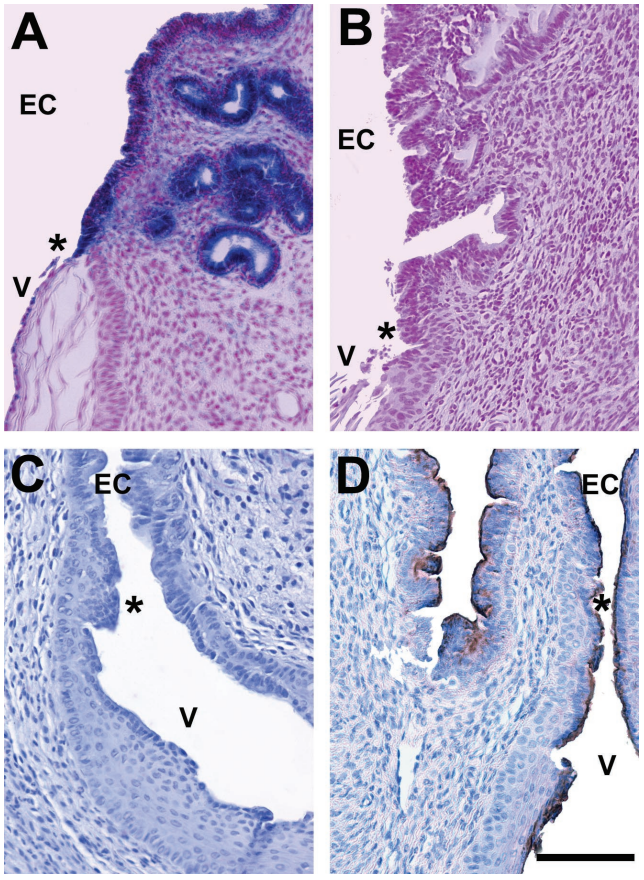

FIG. 1. Cell-specific expression of Fut2-LacZ reporter gene activity in endocervical glandular cells and loss of α(1,2)fucosylated glycans from the lower reproductive tract of Fut2-LacZ-null mice. Fut2-LacZ-null and C57BL/6J wild-type mice were processed for X-Gal staining (top panels) and UEA-I lectin staining (bottom panels). Specific nuclear X-Gal staining was detected in endocervical glandular epithelium of Fut2-LacZ-null mice in estrus (A), while staining in wild-type mice was negative (B). UEA-I lectin staining was absent from Fut2-LacZ-null mice (C) but was associated with endocervical and upper vaginal epithelia of wild-type mice (D), indicating that *Fut2* is essential for expression of lower reproductive tract α(1,2)fucosylated glycans. Endocervical (EC) and vaginal (V) epithelium are marked. The asterisk indicates the location of the squamocolumnar epithelial junction of the cervix. Bar, 100 μm.

4 days displayed hyphal yeast and neutrophils throughout the vagina, confirming *C. albicans* infection (data not shown). Similar to human nonsecretors, Fut2-LacZ-null mice displayed an increased vaginal fungal burden compared to that in wild-type mice of 4.8- and 2.4-fold at 4 and 7 days postinoculation, respectively (Fig. 2). Analysis of variance (ANOVA) determined that the higher colonization rates in Fut2-LacZ-null mice compared to those in wild-type mice cannot be accounted for by expected variation. Upon post-hoc analysis using Tukey's honestly significant difference test and Dunnett's T3 test, Fut2-LacZ-null mice had statistically significant increased susceptibility ($P < 0.01$) versus wild-type mice at 4 and 7 days postinoculation. While displaying the same trend seen at earlier time points, there was an absence of statistical significance at 14 days postinoculation, perhaps as a result of the limited number of mice used in the study. There was no variation

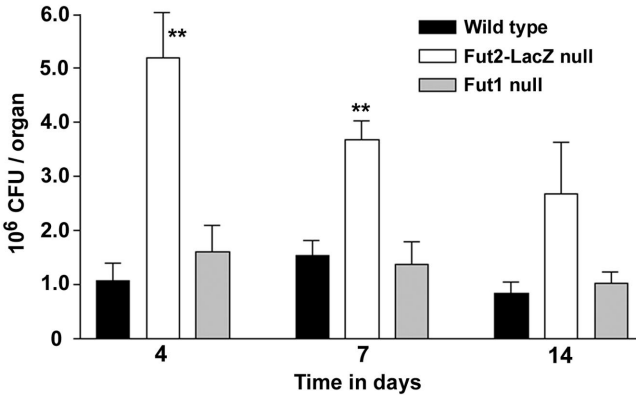

FIG. 2. Quantification of experimental *C. albicans* vaginitis in three mouse genotypes. Fut2-LacZ-null mice, Fut1-null mice, and wild-type mice were subjected to *C. albicans* vaginitis. The experiment was performed in triplicate with similar results; cumulative data are shown ($n = 7$ to 8 mice per group). One-way ANOVA and post-hoc analysis revealed that Fut2-LacZ-null mice display an increased susceptibility (**, $P < 0.01$) versus wild-type mice at 4 and 7 days postinoculation. Error bars show the standard errors of the means.

difference in fungal burden between the control Fut1-null and C57BL/6J wild-type mice.

Neoglycoprotein competitive inhibitors to yeast colonization. To address the mechanism of *Fut2* involvement during *C. albicans* infection, we coinoculated mice with *C. albicans* and exogenous neoglycoproteins to directly saturate adhesin binding sites and potentially reduce colonization by sequestering, and thereby preventing, adhesion to host epithelium (25). Groups of pseudoestrous wild-type and Fut2-LacZ-null mice were intravaginally inoculated with a total of 13 μl composed of 10 μl of 5×10^5 stationary-phase *C. albicans* cells preincubated for 15 min with 3 μl of a 10-mg/ml solution of either bovine serum albumin (BSA), fucose-conjugated BSA (Fuc-BSA), or galactose-conjugated BSA (Gal-BSA) (EY Laboratories, Inc., San Mateo, Calif.). The vagina and cervix were

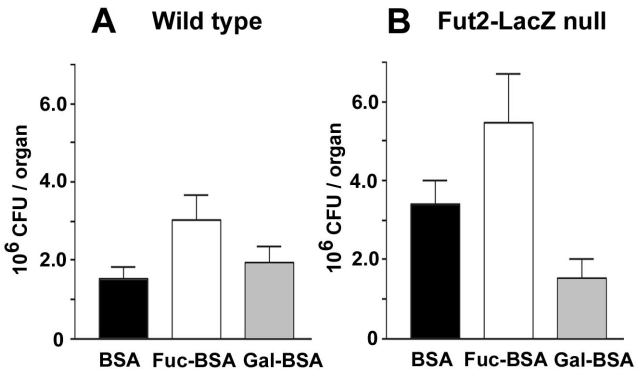

FIG. 3. Effects of neoglycoproteins on susceptibility of Fut2-LacZ-null and wild-type mice to experimental yeast vaginitis. C57BL/6J wild-type (A) and Fut2-LacZ-null (B) mice were inoculated with *C. albicans* cells preincubated with BSA, Fuc-BSA, or Gal-BSA ($n = 9$ to 11 mice per group). Error bars show the standard errors of the means. Under the conditions tested, exogenous neoglycoproteins were not effective in preventing *C. albicans* colonization in Fut2-LacZ-null or wild-type mice.

removed en bloc from all mice 4 days postinoculation, and the CFU per organ was quantified as described above. Comparisons of the mean vaginal fungal burden between treatments in Fut2-LacZ-null and wild-type control mice did not show statistically significantly effects from Fuc-BSA or Gal-BSA pretreatments compared to BSA alone, based on a one-way ANOVA and Dunnett's T3 post-hoc test (Fig. 3). Possible explanations include an inability of simple neoglycoproteins to adequately bind *C. albicans* adhesins, insufficient dosing with the competitive sugars due to rapid turnover or elimination, or an inappropriate growth phase of *C. albicans*. Future studies will include more complex oligosaccharides and lectins that may show greater efficacies to sequester yeast and prevent adhesion to host epithelium. These data provide evidence that Fut2-LacZ-null mice may serve as an animal model to investigate host-microbe interactions in the gastrointestinal and genitourinary systems, where epidemiological data support an association between ABO and Lewis blood group secretion and genetic predisposition to infection.

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