Gastrointestinal mucins of Fut2-null mice lack terminal fucosylation without affecting colonization by Candida albicans

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Introduction

Mucins are the main component of the mucus layer produced by secretory cells lining the respiratory, reproductive, and gastrointestinal (GI) tracts. These high molecular mass, hydrophobic O-linked glycoproteins have been implicated in several important biological processes including host–pathogen interactions, inflammation, lubrication, and cancer metastasis (Hollingsworth and Swanson, 2004). Composition of the mucus layer is affected by spatial and temporal regulation of mucin genes along the length of the GI tract (Gendler and Spicer, 1995) and by exogenous factors such as colonization with commensal and temporary pathogenic organisms. To prevent overcolonization while maintaining a healthy GI tract, oligosaccharides comprise 50–80% of mucin mass which protects against degradation and provides binding sites for commensal and pathogenic organisms, mimicking those expressed by epithelia, thereby promoting their removal with the mucus flow.

Posttranslational synthesis and modification of protein and lipid glycans is determined by specific glycosyltransferases including the α(1,2)fucosyltransferase encoded by FUT2 (secretor) gene, which catalyzes the addition of terminal α(1,2)fucose residues on mucins. Mutation of this gene, which affects ~20% of the human population, is associated with different susceptibilities to GI infections by Norwalk virus (Lindesmith et al., 2003), Campylobacter jejuni (Ruíz-Palacios et al. 2003), and Helicobacter pylori (Ikehara et al. 2001).

Normally Candida albicans is a commensal organism of the intestine and adheres to intestinal mucins without overgrowth or invasion (de Repentigny et al., 2000). We hypothesize that changes within the intestinal microenvironment, such as alterations in specific glycans expressed on mucins, may lead to increased colonization and ultimately invasion of the intestinal epithelia. Fucose has been implicated in C. albicans adhesion in vitro (Cameron and Douglas, 1996; Vardar-Ulnu et al., 1998) and α(1,2)fucosylated glycans expressed by cervical mucins is associated with susceptibility to vaginal candidiasis in Fut2-LacZ-null mice in vivo (Hurd and Domino, 2004). As intestinal C. albicans has been suggested to be a potential reservoir for vaginal candidiasis, we sought to determine whether Fut2 expression could affect C. albicans colonization of the intestine. In this study, we determined the cellular expression of Fut2 in the GI, alterations in mucin O-glycosylation, and tested the role of α(1,2)fucosylated glycans in intestinal C. albicans colonization.

Key words: fucosyltransferase/mucin/O-glycosylation/Secretor gene/yeast

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Results

Fut2-LacZ activity in secretory cell types of the GI tract

The cellular expression pattern of Fut2 within the GI tract was addressed in Fut2-LacZ-null mice using an X-gal staining method as previously described (Domino and Hurd, 2004). Initial assessment of X-gal staining along the entire length of the GI tract at the whole organ level (Figure 1, left panels) revealed intense blue staining within the antrum, cecum, proximal, and distal colon, which was absent in wild-type controls (data not shown). Tissues which displayed X-gal staining at the whole organ level were examined for cellular localization and histology (Figure 1, right panels). Fut2-LacZ expression was observed within Brunner’s glands of the duodenum, and mucus-secreting goblet cells of the cecum, proximal, and distal colon. However, no measurable staining was observed in goblet cells in the small intestine. In addition to X-gal staining, similar staining was detected by immunohistochemistry with a polyclonal β-galactosidase antibody (data not shown).

Fut2 is responsible for α(1,2)fucosylation within the GI tract

To investigate whether other α(1,2)fucosyltransferases may compensate for the loss of Fut2 within the GI tract of Fut2-LacZ-null mice, tissue sections processed for X-gal staining were subsequently stained with Aleuria aurantia agglutinin (AAA) lectin, which broadly detects L-fucosylated glycans. In tissues of wild-type mice, intense brown AAA-lectin staining was observed in mucus-secreting foveolar pit and chief cells of the antrum and goblet cells of the proximal and distal colon (Figure 2, left panels). In contrast, no lectin staining was detected in these tissues from Fut2-LacZ-null mice (Figure 2, right panels).

Absence of terminally fucosylated neutral, sulfated, and sialylated O-linked oligosaccharides within the colon of Fut2-LacZ-null mice

Because the LacZ staining localized to mucin producing cells, the effect of the absence of the Fut2 enzyme on mucin

Fig. 1. Whole organ and tissue-specific examination of X-gal staining within the gastrointestinal tract of Fut2-LacZ-null mice. Specific X-gal staining was observed within antral-duodenal junction (A), cecum (C), proximal (E), and distal colon (G) from Fut2-LacZ-null mice and photographed at 10-fold magnification. Histological analysis of these tissues showed specific X-gal staining was most intense in Brunner’s glands of the duodenum (B), and goblet cells of the cecum (D), proximal (F), and distal (H) colon.

Fig. 2. Microscopic view of Fut2-LacZ-null and C57BL/6J tissues isolated from the gastrointestinal tract stained with Aleuria aurantia agglutinin. Intense brown lectin staining was associated with foveolar pit and chief cells of the antrum (A) and goblet cells of the proximal (C) and distal (E) colon of wild-type mice which was absent, respectively, in antrum (B), proximal (D), and distal (F) colon of Fut2-LacZ-null mice.
mucins, largely made up of Muc2 (Karlsson et al., 1998) and Fut2-LacZ-null mice lack α(1,2)fucosylated glycans on insoluble colonic mucins (Table I). We examined GI colonization by C. albicans in male Fut2-LacZ-null and C57BL/6J wild-type mice. Following inoculation with C. albicans by gavage, daily fecal samples were collected to monitor rates of colonization and to establish euthanization time points for complete analysis of the GI tract (data not shown). Highest fungal burden was observed 7 days after inoculation. As a result, groups of mice were euthanized 7 days after inoculation, their GI tracts removed en bloc and separated into small intestine, cecum, and colon. Statistical analysis, using repeated measures ANOVA, of colony forming units (CFU) obtained from small intestine, cecum, and colon revealed no differences in C. albicans colonization between wild-type and Fut2-LacZ-null mice euthanized at day 7 (Table II). X-gal staining of the small and large intestine was not significantly affected by colonization with C. albicans (data not shown).

Discussion

The mouse Fut2 fucosyltransferase is the orthologue of the human Secretor gene. This enzyme is found in ~80% of the human population, with ethnic variations, and catalyzes the formation of blood group H antigens in mucosal secretions. In this study, utilizing targeted mice with Fut2 replaced by the lacZ gene, the normal expression of the Fut2 gene was relatively straight forward to analyze across the entire GI tract. The highest level of expression was found in mucus-secreting foveolar pit and chief cells of the antrum (Domino and Hurd, 2004), in the Brunner glands, and in the goblet cells of the large intestine. These localizations, including the lack of detectable expression in the small intestine, is consistent with previous studies and suggests that the predominant expression of Fut2 is in cells that produce mucins carrying blood group H(0) epitopes.

We have previously shown that the guanidinium chloride insoluble mucins, largely Muc2, of the caecum and colon have abundant oligosaccharides with Fuc bound as predicted for the specificity of the secretor enzyme (Thomsson et al., 2002). Most of the oligosaccharides found in the large intestine of wild-type C57BL/6J mice in this study were previously found in both wild-type and cystic fibrosis mice (lacking a functional CFTR), except for the sulfated compounds that could not be analyzed previously (Thomsson et al., 2002). In contrast to the rat (Karlsson et al., 1997), the large intestine of mice express only small amounts (5%) of sialylated oligosaccharides as also shown in a previous study using a different analytical approach (Thomsson et al., 2002).

The total absence of Fuc on any of the mucin oligosaccharides in the Fut2-LacZ animal is consistent with the

Fig. 3. Total mass chromatograms of neutral and sulfated O-linked oligosaccharides released from colon insoluble mucins of C57BL/6J wild type (A) and Fut2-LacZ-null mice (B). The B chromatogram did not show any additional peaks when analysis was performed for longer times (not shown). Peaks marked by asterisk indicate impurities within the peak in addition to trace amounts of the oligosaccharide, precluding calculation of relative abundances.

oligosaccharides was examined. The insoluble colonic mucins, largely made up of Muc2 (Karlsson et al., 1996; Thomsson et al., 2002), were isolated from wild-type and Fut2-LacZ-null mice, the O-linked oligosaccharides released and analyzed by mass spectrometry (liquid chromatography electrospray ionization mass spectrometry [LC-ESI-MS] and liquid chromatography electrospray ionization tandem mass spectrometry [LC-ESI-MS/MS]). Seventeen different oligosaccharides with up to eight sugar residues were revealed as their [M – H]– ions (Figure 3 and Table I). Out of these, 11 were neutral, five sulfated and one sialylated. Components based on the core 2 (Galβ1–3GlcNAcβ1–6GalNAc-) sequence were most abundant, but core 1 (Galβ1–3GalNAc-) structures were also present. The structures found are similar to the ones found on insoluble mucins in the colon and small intestine of C57BL/6 mice (Holmen et al., 2002; Thomsson et al., 2002). Most of the oligosaccharides in the wild-type mice mucins, except the shortest precursor compounds, have terminal fucose residues forming the blood group H-type epitope. In contrast, all the peaks for oligosaccharides carrying blood group H-type epitopes were absent in the Fut2-LacZ-null
Loss of mucin fucosylation in Fut2-LacZ-null mice

Table I. Proposed structures of neutral, sulfated and sialylated O-linked oligosaccharides of colonic insoluble mucins from wild-type C57BL/6J and Fut2-LacZ-null mice

<table>
<thead>
<tr>
<th>No.</th>
<th>Molecular ion [M – H]</th>
<th>Sequence/compositiona</th>
<th>Retention time (min)</th>
<th>Relative abundance (%)b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Wild type</td>
<td>Fut2-LacZ-null</td>
</tr>
<tr>
<td>N2.1</td>
<td>384</td>
<td>Gal-3GalNAcol</td>
<td>16.4</td>
<td>Trace</td>
</tr>
<tr>
<td>N3.1</td>
<td>587a</td>
<td>Gal-3(GlcNAc-6)GalNAcol</td>
<td>19.6</td>
<td>11</td>
</tr>
<tr>
<td>N3.2</td>
<td>587b</td>
<td>Gal, HexNAc, GalNAcol</td>
<td>20.2</td>
<td>—</td>
</tr>
<tr>
<td>N3.2</td>
<td>587c</td>
<td>Gal, HexNAc, GalNAcol</td>
<td>21.9</td>
<td>—</td>
</tr>
<tr>
<td>N3.3</td>
<td>530</td>
<td>Fuc-2Gal-3GalNAcol</td>
<td>31.9</td>
<td>46</td>
</tr>
<tr>
<td>N4.1</td>
<td>749a</td>
<td>Gal-3(Gal-3/4GlcNAc-6)GalNAcol</td>
<td>22.7</td>
<td>—</td>
</tr>
<tr>
<td>N4.2</td>
<td>749b</td>
<td>Gal-3(Gal-4/3GlcNAc-6)GalNAcol</td>
<td>23.0</td>
<td>—</td>
</tr>
<tr>
<td>N4.3</td>
<td>749c</td>
<td>Gal-4GlcNAc-3Gal-3GalNacol</td>
<td>23.6</td>
<td>—</td>
</tr>
<tr>
<td>N4.4</td>
<td>733</td>
<td>Fuc-2Gal-3(GlcNAc-6)GalNAcol</td>
<td>32.4</td>
<td>16</td>
</tr>
<tr>
<td>N6.1</td>
<td>1041a</td>
<td>2 Fuc, 2 Gal, HexNAc, HexNAcol</td>
<td>32.6</td>
<td>2</td>
</tr>
<tr>
<td>N6.2</td>
<td>1041b</td>
<td>Fuc-2Gal-3(Fuc-Gal-GlcNAc-6)GalNAcol</td>
<td>33.7</td>
<td>5</td>
</tr>
<tr>
<td>Su4.1</td>
<td>667</td>
<td>Gal-3(SO₃⁻/4/6GlcNAc-6)GalNAcol</td>
<td>23.1</td>
<td>Trace</td>
</tr>
<tr>
<td>Su5.1</td>
<td>829a</td>
<td>Gal-3(Gal-3/4(SO₃⁻/4/6)GlcNAc-6)GalNAcol</td>
<td>25.4</td>
<td>—</td>
</tr>
<tr>
<td>Su5.2</td>
<td>829b</td>
<td>Gal-3(Gal-4/3(SO₃⁻/4/6)GlcNAc-6)GalNAcol</td>
<td>26.9</td>
<td>—</td>
</tr>
<tr>
<td>Su5.3</td>
<td>813</td>
<td>Fuc-2Gal-3(SO₃⁻/4/6)GlcNAc-6)GalNAcol</td>
<td>36.4</td>
<td>17</td>
</tr>
<tr>
<td>Su7.1</td>
<td>1121</td>
<td>Fuc-2Gal-3(Fuc-(SO₃⁻/4/6)GlcNAc-6)GalNAcol</td>
<td>36.5</td>
<td>2</td>
</tr>
<tr>
<td>Si8.1</td>
<td>1469</td>
<td>SO₃⁻, NeuAc, 1 Fuc, 2 Gal, 2 HexNAc, HexNAcol</td>
<td>35.5</td>
<td>Trace</td>
</tr>
</tbody>
</table>

Compositions and sequences were elucidated by LC-ESI-MS and LC-ESI-MS/MS. N, neutral; Si, sialylated; Su, sulfated.

aThe saccharides marked in bold are located on C-6 of GalNAcol. The following assumptions have been made due to previously published data (Thomsson et al., 2002): hexose residues are Gal, deoxyhexose residues are Fuc, N-acetylhexosamine linked to GalNAcol is GlcNAc, and N-acetylhexosaminitol residues are GalNAcol. For the sulfated oligosaccharides, the sulfate group is suggested to be located on the GlcNAc residue on the C-6 branch of GalNAcol according to precursor structures and previously published data (Thomsson et al., 2000; Robbe et al., 2004). The nonsubstituted GalNAcol was not analyzed.

bMeasured as LC-MS chromatogram peak areas divided with the total peak areas of the specific sample, expressed as percentage. Trace amounts were not used in the relative abundance calculation. Oligosaccharides marked — were not detected in the sample.

Table II. Lack of difference in fungal burden in gastrointestinal of wild type and Fut2-LacZ-null mice 7 days after gavage with Candida albicans

<table>
<thead>
<tr>
<th>Genotype (n = 14)</th>
<th>Mean CFU/g recovered from small intestine ± SEM</th>
<th>Mean CFU/g recovered from cecum ± SEM</th>
<th>Mean CFU/g recovered from colon ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>7.2 ± 1.5</td>
<td>30.3 ± 6.9</td>
<td>30.0 ± 6.4</td>
</tr>
<tr>
<td>Fut2-LacZ null</td>
<td>4.6 ± 1.2</td>
<td>32.4 ± 8.0</td>
<td>30.8 ± 8.0</td>
</tr>
</tbody>
</table>

Fut2 enzyme being the predominant fucosyltransferase expressed in the mouse intestine. This is in contrast to humans, where a majority of the population also carries the Lewis epitopes in the intestine. The oligosaccharide structures found in Fut2-LacZ-null mice, in contrast to wild-type animals, were all consistent with the accumulation of Fut2 precursor compounds. Thus we could not observe any additional oligosaccharides because of compensation by induction of fucosyl- or sialyltransferases.

Fut2 enzyme has been previously shown to undergo dynamic regulation of its expression in studies of the small intestine of germ-free mice inoculated with Bacteroides thetaiotaomion (Bry et al., 1996; Lin et al., 2001), in cystic fibrosis (CF) mice (Thomsson et al., 2002), and with an intestinal infection caused by the parasite Nippostrongylus brasiliensis (Holmen et al., 2002). The parasite caused a transient induction of the Fut2 enzyme in the middle of the 12-day infectious cycle. In CF animals, there was a dramatically increased expression of the Fut2 transcript and the concomitant blood group H product in the small intestine. The reason for this is not fully understood, but it was suggested that this was due to an increased load of bacteria in the CF mice small intestine. This assumption was recently substantiated by microbiological studies (Norkina et al., 2004). The results in the this study suggest that while the expression of the Fut2 gene is sensitive to microbial and parasitic colonization of the small intestine, fungal infection did not have such an affect.

In contrast to vaginal inoculation of C. albicans, colonization of the G1 tract after gavage did not differ significantly in the Fut2-LacZ-null mice compared to wild-type controls. Possible explanations include differences in innate immunity between the G1 and lower reproductive tract or differences in dose–response and timing of the infection. The data from this mouse model suggest that the increased susceptibility of nonsecretor women to C. albicans vaginitis...
might thus be more due to a local effect in the vagina than to an increased colonization of their intestine.

Materials and methods

Animals
Male C57BL/6J (Jackson Laboratory, Bar Harbor, ME; stock no. 000664) and Fut2-LacZ mutant mice (Domino et al., 2001), backcrossed for 12 generations to C57BL/6J (designated B6.129X1-Fut2m7m1S60), 8–10 weeks of age were maintained under specific pathogen free conditions and handled according to institutionally recommended guidelines.

Histological staining
Fut2-LacZ-null and C57BL/6J mice were terminally perfused with ice-cold phosphate-buffered saline (PBS) followed by 4% paraformaldehyde. GI tracts were removed and partially fixed in ice-cold 4% paraformaldehyde for 20 min on ice. For X-gal staining, tissues were washed three times in 0.1-M sodium phosphate pH 7.3 and stained overnight with X-gal at 37°C as described previously (Domino and Hurd, 2004). Whole organs were either photographed or postfixed in 10% buffered formalin and 0.2% glutaraldehyde overnight, paraffin embedded, sectioned, and stained with nuclear fast red by Histoserve (Gaithersburg, MD), using substitutes for organic solvents to preserve the X-gal staining. Tissue blocks which showed significant X-gal staining were subsequently resectioned, processed with biotin conjugated Aleuria aurantia agglutinin (EY Laboratories, San Mateo, CA) and counterstained with hematoxylin by the University of Michigan IPOX histology core. Polyclonal β-galactosidase IgG fraction conjugated to biotin was purchased from Rockland Immunochemicals (Gilbertsville, PA).

Isolation of intestinal insoluble mucins, release and analysis of O-linked oligosaccharides
Insoluble intestinal mucins were isolated from 10 C57BL/6J and Fut2-LacZ-null mice as described previously (Thomsson et al., 2002). Briefly, pooled mucosas were scraped into 6-M guanidium chloride. Insoluble mucins were recovered by centrifugation and the pellets washed and solubilized by reduction followed by alklylation and dialysis. The O-linked oligosaccharides from the insoluble mucins (~100 µg) were released with 0.05-M potassium hydroxide and 0.5-M sodium borohydride (Carlstedt et al., 1993) but without separation into fractions. The oligosaccharides were desalted on a column of cation exchange resin AG50Wx8 (BioRad Laboratories, Hercules, CA). The O-linked oligosaccharides were eluted by LC-ESI-MS and LC-ESI-MS/MS. The oligosaccharides were dissolved in water and separated on a Hypercarb (5-µm particles) column (15 cm × 0.32 mm) (Thermo-Hypersil, Runcorn, UK) at a flow rate of 5 µl min⁻¹. A 10-mM ammoniumbicarbonate-acetonitrile gradient (0–40% acetonitrile) was used. The column was coupled to a quadrupole orthogonal acceleration time-of-flight mass spectrometer (Q-Tof; Micromass, Manchester, UK), operated in the negative ion mode. The electrospray voltage applied was –3.5 kV. For LC-ESI-MS/MS, [M – H] ions were collided with argon as collision gas with a collision energy increasing from 22 eV to 60 eV for m/z 300 to m/z 2000.

GI inoculation with C. albicans

C. albicans (3153A), originally a clinical isolate now propagated in the laboratory, was grown to stationary phase in 1% phytone peptone (Becton Dickinson, Cockeysville, MD) supplemented with 0.1% glucose for 16–18 h at 30°C in an orbital shaking incubator. The culture was washed in PBS and quantified using a hemocytometer. Fut2-LacZ-null and C57BL/6J male mice were inoculated with ~2 × 10⁷ stationary-phase C. albicans (3153A) by gavage in a 100-µL volume using a 1-mL syringe equipped with gavage needle. Fecal samples were collected daily, weighed and immediately homogenized in 100-µL sterile PBS. Samples were serially diluted on chloramphenicol supplemented Sabouraud dextrose agar plates and incubated at 35°C for 48 h, after which CFU were determined. At day 7 post-infection, mice were killed, and GI tracts removed en bloc and separated into small intestine (from antral–duodenal junction to ileo–cecal junction), cecum and colon (from cecal junction to rectum). Following weighing and homogenization in PBS, samples were serially diluted and plated on chloramphenicol supplemented Sabouraud dextrose agar plates and incubated at 35°C for 48 h, after which CFU were determined (Mellado et al., 2000). In addition, X-gal staining was performed on GI tissues of Fut2-LacZ-null mice 7 days after gavage with C. albicans versus PBS alone.

Statistics
Comparisons between groups were made using repeated measures analysis of variance (ANOVA) using the program SPSS 12. Statistical significance was defined as a P value of <0.05.

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Abbreviations
CF, cystic fibrosis; CFU, colony forming units; Fut2, α(1,2)fucosyltransferase “Secretor” gene; GI, gastrointestinal; LC-ESI-MS, liquid chromatography electrospray ionization mass spectrometry; LC-ESI-MS/MS, liquid chromatography electrospray ionization tandem mass spectrometry; PBS, phosphate-buffered saline.
References


