Human Milk Oligosaccharides Inhibit Candida albicans Invasion of Human Premature Intestinal Epithelial Cells\textsuperscript{1,2}

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Abstract

Background: Human milk oligosaccharides (HMOs) are a highly abundant, diverse group of unique glycans that are postulated to promote the development of a protective bacterial microbiota in the intestine and prevent adhesive and invasive interactions of pathogenic bacteria with mucosal epithelia. Candida albicans, a prevalent fungal colonizer of the neonatal gut, causes the majority of fungal disease in premature infants and is highly associated with life-threatening intestinal disorders.

Objective: The objective of the current study was to test the hypothesis that HMOs protect human premature intestinal epithelial cells (pIECs) from invasion by C. albicans.

Methods: To study fungal invasion, a quantitative immunocytochemical assay was used to distinguish invading from noninvading C. albicans cells in the presence and absence of HMOs. To understand how HMOs affect C. albicans invasion of pIECs, the expression of C. albicans virulence traits that are important for invasiveness (hyphal morphogenesis and ability to associate with host cells) were quantified.

Results: Treatment with HMOs reduced invasion of pIECs by C. albicans in a dose-dependent manner by 14–67\%, with a physiologic concentration (15mg/mL) of HMOs causing a 52\% reduction in invasion (P < 0.05). The decreased invasive ability of C. albicans was associated with hyphal lengths that were \textbf{\textit{30\% shorter}} (P < 0.05), likely because of a delay in the induction of hyphal morphogenesis after inoculation of yeast onto pIECs, which correlated with a 23\% reduction in the combined expression level of hyphal-specific genes (P < 0.05). In addition, HMOs caused a 40\% decrease in the number of C. albicans cells able to associate with pIECs at the time of hyphal induction (P < 0.05).

Conclusions: These results, obtained with the use of a primary pIEC model, indicate that HMOs reduce virulence characteristics of C. albicans and suggest a role for HMOs in protecting the premature infant intestine from invasion and damage by C. albicans hyphae. J Nutr 2015;145:1992–8.

Keywords: Candida albicans, fungal invasion, fungal pathogenesis, human milk oligosaccharides, hyphal morphogenesis, intestinal epithelial cells, premature infants

Introduction

Breast milk feeding is associated with numerous documented benefits to infants compared with formula feeding. These potential advantages are especially important for premature infants and include lower rates of infection and intestinal disease, with the potential for improved neurodevelopmental outcomes (1, 2). The identification of the specific components of breast milk that confer health benefits has been the focus of much research. One molecular component, oligosaccharides, provides substrates in the intestine that can be metabolized only by certain bacteria, thereby promoting their growth (3). These bacteria, notably Bifidobacteria and Bacteroides, are associated with beneficial health effects; thus, administration of milk oligosaccharide preparations has been proposed as a prebiotic strategy to reduce disease in premature infants (4).

Human milk oligosaccharides (HMOs)\textsuperscript{6} are complex unconjugated glycans of diverse structure that comprise the third-largest fractional component of breast milk. Over one hundred unique oligosaccharides have been identified in human milk.

\textsuperscript{6} Abbreviations used: ACT\textsubscript{1}, yeast gene encoding actin 1; ALS3, yeast gene encoding agglutinin-like sequence 3; ECE1, yeast gene encoding extent of cell elongation 1; HGC1, yeast gene encoding hypha-specific expression and relatedness to G1 cyclins; HMO, human milk oligosaccharide; HSG, hyphal-specific gene; HWI, yeast gene encoding hyphal wall protein 1; HYRE, hyphally regulated yeast gene 1; pIEC, premature intestinal epithelial cell; SAE5, yeast gene encoding secreted aspartyl proteinase 5; SAP6, yeast gene encoding secreted aspartyl proteinase 6; SDC, synthetic dextrose complete.
Colostrum, the early milk produced after birth, contains as much as 20–25 g HMOs/L, whereas mature breast milk usually contains lower concentrations of 5–20 g/L (3). In the first ~2 mo of life, the majority of HMOs reach the intestine and are excreted in feces largely intact, with compositions that are similar to those of samples taken from breast milk (5, 6). This result suggests that HMO-metabolizing bacteria are not yet present in substantial numbers in the young infant’s intestinal tract. As infancy progresses, HMOs become progressively more digested, eventually disappearing from intestinal samples as foods other than breast milk are added to the diet. In addition to their aforementioned proposed prebiotic activity, HMOs also modulate intestinal epithelial cell growth (7, 8), inhibit adhesive and invasive interactions of pathogenic microbes with the intestine (9, 10), and reduce the incidence of disease in a rat model of necrotizing enterocolitis (11). These broad-based activities of HMOs make them attractive candidates as dietary supplements to improve infant health.

Disseminated candidiasis is a frequent life-threatening infection in premature infants with rates as high as 23% in those born at extremely low birth weight (<1000 g) (12). Candida species are prominent fungal colonizers of the infant intestine (13–15), where invasion often leads to dissemination and systemic infection (16–18). Candida albicans sepsis often occurs concurrently in infants with fragile intestinal barriers because of gastrochisis, necrotizing enterocolitis, intestinal perforation, and extreme prematurity (19, 20), highlighting the importance of fungal–intestinal interactions in the pathogenesis process. For necrotizing enterocolitis and intestinal perforation, it has been proposed that C. albicans itself may contribute to the initial intestinal injury that leads to these disorders (21). This hypothesis is supported by in vitro studies showing that C. albicans is able to penetrate, injure, and cause inflammatory cytokine release from human premature intestine epithelial cells (pIECs) (22), phenotypes associated with the unique ability of C. albicans, with respect to other Candida species, to form a filamentous hyphal morphology. Therapies that reduce C. albicans colonization and prevent pathologic fungal–host cell interactions within the intestine thus have the potential to improve intestinal barrier function and prevent fungal-associated morbidity and mortality in premature infants, as well as in other immunocompromised patient populations. To date, only a few studies have explored the antifungal effects of human milk, the majority of which have focused on the benefits of milk macrophages (23–25). Thus, the goal of the current study was to explore the ability of HMOs to inhibit fungal interactions with the premature infant intestine that are important for disease pathogenesis.

Methods

Isolation and preparation of oligosaccharides from human milk

Pooled HMOs were prepared with the use of centrifugation, filtration, and chromatography as previously described (11) and lyophilized for long-term storage. Milk from 36 different donors was pooled to account for heterogeneity in HMO composition between different women. The human milk donation program has been reviewed by the institutional review board at the University of California, San Diego, and certified as exempt from institutional review board review under Code of Federal Regulations 45 CFR 46.101(b), category 4, because subjects cannot be identified and linked to generated data. HMO composition was analyzed by HPLC and MS to ensure relative consistency of HMO composition among batches. The pooled preparation contained 32.7% lacto-N-tetraose, 19.2% 2’fucosyllactose, 15.8% lacto-N-fucopentaose I, 6.6% difucosyllacto-N-tetraose, 5.7% 3’ sialyllactose, 4.3% lacto-N-neotetraose, 2.9% lacto-N-fucopentaose II, 2.6% lacto-N-tetraose c, 2.4% 3-fucosyllactose, 1.6% sialylacto-N-tetraose b, 1.4% lacto-N-fucopentaose III, 1.1% disialylactose-N-tetraose, and several other HMOs with <1% relative abundance. Lyophilized HMOs were weighed, diluted into fresh tissue culture (H4) media (26) at the concentrations noted, and stored at 4°C.

Cell growth and maintenance

pIECs (H4 cell line) were propagated and maintained as previously described (26). C. albicans strains SC5314 (27), A022b (22), and A003 (22) were recovered from a 15% glycerol frozen stock by plating onto Yeast Peptone Dextrose agar (28) and incubating at 30°C. Fungal cells were propagated for experiments by inoculation of single colonies into synthetic dextrose complete (SDC) liquid media containing 2% glucose (28) and incubated overnight at 30°C. Concentrations of fungal cells were determined visually with the use of a hemocytometer and then subcultured into H4 tissue culture media for use in experimental assays.

pIEC invasion assay

Invasion assays were performed as previously described (22, 29). Briefly, H4 pIECs were infected with 2 × 10^5 C. albicans suspended in H4 media, with or without the addition of HMOs, and incubated for 3 h. In general, yeast cells are noninvasive at any time point with respect to the H4 cell line (22). For fungal hyphae, the 3 h time point was when hyphal invasion of H4 cells could be definitively visualized and quantified. Before this time, hyphae are too short to make a determination of invasiveness. After 3 h, infected pIECs were fixed and fungal invasion was determined immunocytochemically. Noninvading fungal cells became fluorescently tagged by this procedure, whereas invading fungal cells are inaccessible to the primary antibody and are not tagged. Approximately 30 fungal cells were analyzed for each experimental condition and day.

Vegetative yeast growth assays

Agar growth. Yeast cells were grown overnight as described above, and counted and normalized to 1 × 10^6 cells/mL in fresh SDC medium. Tenfold serial dilutions were prepared at concentrations from 10^6 to 10^7 μl of each dilution was spotted onto SDC agar plates onto which had been spread 100 μl H4 media with or without 15 g pooled HMOs/L. Plates were incubated at 30°C for 24 h, and then growth was visualized by direct inspection.

Growth rates in liquid medium. Yeast cells were grown overnight as described above, counted, and normalized to 1 × 10^6 cells/mL. One hundred microliters of cells in H4 media (either with or without the addition of 15 g HMOs/L) was inoculated into wells of a 96-well plate and incubated at 28°C. Cell concentrations were quantified spectrophotometrically, with absorbance readings taken at 600 nm with a Tecan instrument and Magellan v7.1 software (Tecan Group) every 15 min for 24 h. Readings were analyzed with the use of MATLAB R2012b (MathWorks) and the Plot96v2 script (revised from Lawless et al. (30)) to fit a function (logistic/sigmoid or polynomial) to the raw spectrophotometric data and then infer doubling times at the time of most rapid growth from the resulting curves. Doubling times were reported as means of 6 determinations for each condition, time point, and day.

Analysis of fungal morphogenesis

Morphometry. C. albicans cells were incubated with pIECs as described above for the invasion assay for 45 min, 60 min, 75 min, and 3 h. After the indicated incubation times, cells were fixed with 4% paraformaldehyde for 10 min and mounted onto glass slides, and fungal morphologies were visualized by differential interference contrast microscopy. Lengths and widths of fungal hyphae were measured with the use of the line tool of MetaMorph version 6.3r7 (Molecular Devices). The length measurement was taken from the base to the tip of the hypha; width was measured at the widest point of the hypha, determined visually. Lengths and length-to-width ratio were calculated for ~30 cells for each condition, time point, and day.

HMOs protect host cells from C. albicans infection
Quantification of hyphal induction efficiency. With the use of the images obtained as described above, the number of cells forming hyphae was quantified at each time point after incubation with pIECs. A cell was considered to be forming a hypha if the daughter hypha was at least one-half the length of the mother cell and the mother–daughter junction was not constricted (constricted junctions are more indicative of a yeast bud) (31). The percentage of cells forming hyphae was calculated for ∼30 cells for each condition, time point, and day.

Fungal–host cell association assay
With the use of the images collected from the interaction time course (see morphometry section above), random high-power fields from each condition and time point were chosen for analysis of fungal–pIEC associations. An association was defined as a fungal cell (any morphology) and a pIEC that were present in the same plane of view, with the outlines of each cell type being distinct and clear. In preliminary control experiments, experimental conditions and time points did not differ with respect to the number of fields in which a confluent pIEC monolayer was present (>90% of fields for all conditions and time points); microscopic fields lacking pIECs were not included in the analysis. In addition, there was no statistical difference in the number of yeast cells present per high-power field between HMO and non-HMO conditions (data not shown). The total number of fungal cells associating with pIECs was determined for 30 high-power fields for each condition, time point, and day.

qPCR analysis of hyphal-specific gene expression
pIECs were grown to ∼80% confluence in a 12-well tissue culture format. pIEC monolayers were infected with 2 × 10⁵ C. albicans cells suspended in 1 mL H4 media either containing or lacking HMOs (concentrations as indicated in results) and incubated for 45, 60, and 75 min at 37°C with 5% CO₂. After each incubation, experimental and control wells were scraped with sterile round-end toothpicks to harvest cellular material, which was immediately frozen in liquid nitrogen and stored at −80°C until use. From thawed cells, RNA was isolated and quantified, cDNA was generated from RNA, and qPCR was used to detect and quantify hyphal-specific gene (HSG) expression as previously described (32). Primer sequences for HSGs [yeast gene encoding agglutinin-like sequence 3 (ALS3); yeast gene encoding extent of cell elongation 1 (ECE1); yeast gene encoding hypha-specific expression and relatedness to G1 cyclins 1 (HGC1); yeast gene encoding hyphal wall protein 1 (HWP1); hyphally regulated yeast gene 1 (HYR1); yeast gene encoding secreted aspartyl proteinase 5 (SAP5) and yeast gene encoding secreted aspartyl proteinase 6 (SAP6)] and yeast gene encoding actin 1 (ACT1) were as previously published (33).

Statistical analyses
Statistical analyses were performed with the use of SPSS 17.0 for Windows. To compare means for pIEC invasion, an ANOVA blocked by condition and time point was performed followed by post hoc separation of means with the use of Tukey’s honestly significant difference test. For all other assays, a Student’s t test design pairing the data by experimental day was used to compare only HMO and control conditions within each time point; no statistical comparisons were made between time points. P values ≤ 0.05 were considered to be significant for all statistical analyses.

Results

HMOs reduce pIEC invasion by C. albicans in a dose-dependent manner. HMOs decrease invasive interactions of pathogenic bacteria and parasites with the intestine. To assess whether HMOs also inhibit invasion of the premature intestine by fungi, we quantified the ability of C. albicans to penetrate pIEC monolayers in the presence or absence of physiologic concentrations of HMOs (range: 5–20 g/L) (Figure 1A). A significant reduction in C. albicans invasion of pIECs was observed with the addition of 5 g HMOs/L, with progressively increasing inhibition observed as HMO concentrations increased (Figure 1B). For the aforementioned experiment, HMOs were present from the beginning of the yeast–pIEC incubation. At this time, C. albicans is in the yeast morphology and has not yet formed hyphae. To learn whether HMOs were able to specifically inhibit hyphal invasion, we quantified C. albicans penetration of pIECs in the presence and absence of 15 g HMOs/L, added to the incubation after hyphae had already formed (75 min, “late” HMOs). Addition of “late” HMOs did not inhibit C. albicans hyphae from penetrating pIECs; the amount of penetration was similar to that of incubations lacking HMOs (P > 0.05; data not shown). These results indicate that HMOs at physiologic concentrations present in breast milk and added before invasive hyphal formation are able to inhibit C. albicans invasion of pIECs in vitro.

HMOs inhibit C. albicans hyphal, but not yeast, form growth. To assess whether the inhibitory effect of HMOs on pIEC invasion by C. albicans was associated with HMOs causing decreased fungal viability and propagation, we measured fungal growth in the presence and absence of HMOs. We observed that the addition of 15 g HMOs/L had no effect on the growth of 3 strains of C. albicans either on agar or in liquid media (Figure 2). For fungal growth in liquid medium, doubling times were not significantly different when C. albicans cells were incubated in media containing HMOs compared with in media alone (50.7 ± 8.3 min vs. 49.3 ± 14.5 min, respectively, P = 0.43; data not shown).

To learn whether the inhibitory effect of HMOs on pIEC invasion by C. albicans was associated with their ability to reduce hyphal-form growth, we analyzed C. albicans morphology. We found that hyphal width was not affected by the presence of HMOs (data not shown). In contrast, hyphal lengths were significantly reduced by ∼30% in the incubations containing HMOs compared with incubations containing media alone (Figure 3A). Additionally, when 15 g HMOs/L was added after hyphal initiation, no difference in length was observed (P > 0.05; data not shown). This again supports the idea that HMOs have no significant effect once hyphae have been established, and highlights the hyphal initiation phase as important in our model.
To evaluate whether shorter hyphal lengths were due to a delayed time of initiation vs. an overall slower rate of elongation in the presence of HMOs, we performed a time-course analysis of hyphal growth. For the 45 and 60 min time points, hyphal protrusions were either absent or too short to be accurately measured for both HMO- and non-HMO-containing conditions. At the 75 min time point, hyphal lengths were significantly shorter (by ~30%) in the HMO-containing condition than in the media-alone condition (Figure 3A), similar to the reduction seen at the conclusion of the 3 h incubation assay noted above. Together, these results indicate that treatment with HMOs is associated with shorter hyphal lengths at both early and later time points of fungal–pIEC interaction.

To assess whether the time of initial hyphal emergence differed between HMO and non-HMO conditions, we quantified the number of yeast cells forming short hyphal protrusions at early time points after interaction with pIECs. In conditions lacking HMOs, ~40% of yeast cells had formed visible hyphal protrusions by 60 min (Figure 3B). Treatment of yeast cells with HMOs resulted in a 50% reduction in the number of cells forming hyphae at 60 min (Figure 3B). The delay in hyphal initiation resulting from HMO treatment did not appear to require the presence of epithelial cells because the same delay was observed in tissue culture media lacking pIECs (data not shown). For time points after 60 min, there were no differences observed in the proportions of cells forming hyphae either with or without HMOs (Figure 3B), although, as previously noted, the hyphae were shorter in incubations containing HMOs (Figure 3A). Altogether, these data indicate that HMOs do not inhibit C. albicans hyphal initiation, but rather that HMOs delay the time of hyphal emergence, which results in overall shorter hyphae.

**HMOs reduce the ability of C. albicans to associate with pIECs.** To assess whether or not the inhibitory effect of HMOs on C. albicans invasion of pIECs is associated with a decreased ability of fungal cells to associate with pIECs, we quantified fungal–host cell associations in the presence and absence of HMOs. As expected, with a longer time of incubation, greater numbers of fungal cells were observed to be associating with pIECs for non-HMO conditions (Figure 4, gray bars). At the 60 min time point, HMO treatment resulted in a significant reduction (~40%) in the number of fungal–host cell associations compared with the non-HMO control. At the earlier (45 min) and later (75 min) time points, an inhibitory effect of HMOs on fungal–host cell associations was not observed. Overall, these results are consistent with the idea that HMOs, at physiologic concentrations of 15 g/L, are more effective in reducing pIEC associations of cells starting to form hyphae than those of yeast or fully formed hyphal cells.

**HMOs reduce HSG expression by C. albicans.** To further investigate the effect of HMOs on C. albicans hyphal morphogenesis, we studied the expression of HSGs by C. albicans in the presence and absence of HMOs. We examined the expression of 7 HSGs all previously shown to have increased expression in hyphal than in yeast cells (33). HSG expression was initially measured at 60 min after inoculation of C. albicans to pIECs monolayers because this is the time at which hyphae were just beginning to emerge in our in vitro model of fungal invasion. Four of the 7 HSGs that were evaluated had a significantly reduced expression that was associated with HMO treatment. HYR1, HWPI, and ECE1, which encode hyphal cell wall proteins, as well as ALS3, coding for a hyphal-specific adhesion protein, exhibited reduced expression in the presence of HMOs compared with H4 media alone (Figure 5). When expression of all 7 HSGs was combined, the aggregate expression also showed a significant reduction in the presence of HMOs (Figure 5, left bars), indicating a trend for reduced expression in all the HSGs analyzed. We also evaluated the expression levels of HSGs over time. At 45 min after inoculation to pIECs, HSG induction had not yet occurred, consistent with the observation that yeast cells were not yet forming hyphae (data not shown). At 60 min, C. albicans cells exposed to HMOs had reduced expression of HYR1, HWPI, ECE1, and ALS3 compared with non-HMO–treated control cells, as previously noted (Figure 5). At 75 min, the differences in expression were no longer observed for these HSGs (data not shown). Furthermore, when HMOs were added after hyphae had already formed (75 min after inoculation) rather than at the time of inoculation, no significant difference was seen in HSG expression compared with H4 media alone (P > 0.05; data not shown). Altogether, these results indicate that the inhibitory effect of HMOs on C. albicans morphogenesis is greatest at the time of hyphal initiation/establishment, and are consistent with our previous data showing a delay in hyphal formation when fungal cells are incubated with HMOs (Figure 3).

**Discussion**

Oligosaccharides that are present in human milk are of great interest to medical researchers and practitioners because they have the potential to modulate the intestinal microbiota and protect the intestine from injury and invasion by pathogens. Although HMOs have been shown to inhibit pathogenic interactions between bacteria and parasites and human mucosal epithelia (9, 34, 35), a role for HMOs in inhibiting fungal interactions with the host has not previously been explored. Antifungal prophylaxis with the use of fluconazole has been shown to decrease
intestinal colonization with C. albicans and reduce the incidence of systemic C. albicans infections in extremely premature infants who are hospitalized in units with a high incidence of invasive candidiasis (36). HMOs, being natural molecules, have the potential to avoid the pitfalls exhibited by antifungal antibiotics such as unappreciated side effects, development of antibiotic resistance, and selection for naturally resistant fungi (37). Thus, a use for HMOs in preventing intestinal injury and invasion by fungi warrants investigation.

In this study, we examined how the addition of HMOs affects interactions between the opportunistic fungal pathogen C. albicans and pIECs, a cellular model with high clinical relevance for premature infants. The ability of C. albicans in both yeast and hyphal forms to stably associate with host cells is considered the critical first step for eventual tissue invasion (38). In addition, C. albicans hyphal morphogenesis is thought to facilitate invasion in at least two ways: hyphal elongation provides the active, physical force needed to penetrate host cells and hyphae produce hyphal-specific proteases from their tips that digest components of host cell membranes (39, 40). We found that HMOs impair early fungal–host cell associations, as well as hyphal morphogenesis. In the time-course analyses, HMOs caused a significant reduction in fungal–host cell contacts only at the 60 min time point. The observation that HMOs did not cause decreased cell contacts at later times is unlikely to be due to consumption (i.e., reduction) of HMOs by C. albicans because metabolism of HMOs appears to be a function unique to only some species of bacteria (3). The 60 min time point is the only time at which there was a significant difference in HMO vs. control groups with respect to percentage of cells forming hyphae (Figure 3B). This result suggests that hyphae are better able to make stable contacts with pIECs than yeast-form cells, consistent with the results of a previous study of C. albicans interactions with endothelial cells under static conditions (41, 42). One way that HMOs may cause C. albicans to be less effective at epithelial cell invasion is by interfering with the timely initiation of hyphal morphogenesis. Delayed hyphal morphogenesis, in turn, impairs the establishment of stable pIEC contacts that are needed to facilitate invasion, as well as the ability to actively penetrate host cells. The result that HMOs cannot inhibit already-established hyphae from invading pIECs is consistent with the idea that early cellular interactions, when hyphae are just forming, are important for the pathogenesis process. In addition, it is possible that longer hyphae require more HMOs to physically block C. albicans cell surface structures that are needed for contact with, and invasion of, pIECs, a conclusion that correlates with the HMO dose-response results. In either case, our results indicate that physiologic concentrations of pooled HMOs reduce C. albicans virulence features, with optimal dosing of HMOs associated with clinical benefits yet to be determined.

Addition of HMOs to C. albicans affected hyphal morphogenesis at not only the cellular but also the transcriptional level. We observed that expression of a gene encoding a C. albicans hyphal-specific adhesin, ALS3, was reduced in the presence of HMOs at the 60 min time point. Expression of ALS3 is detected ~30 min after C. albicans cells are transferred into media that induces hyphal morphogenesis and C. albicans mutant strains lacking the Als3 protein adhere less well to buccal epithelial cells at early time points after inoculation than do strains expressing ALS3 (43). Together with our results, these findings support the hypothesis that reduced or delayed ALS3 expression by nascent hyphae leads at least in part to the decreased ability of C. albicans to associate with epithelial cells at early infection times. In addition, it is possible that HMOs physically block sites on the C. albicans cell surface that are needed for the fungus to associate with pIECs at early interaction times. This idea is consistent with the results of previous studies showing that HMOs physically attach to Entamoeba histolytica and cause them to detach from adult human intestinal epithelial cells (9) and reduce adhesion of Campylobacter jejuni to cultured human epithelial cells (35).

Our results indicate that physiologic concentrations of pooled HMOs are able to reduce C. albicans association with, and invasion of, pIECs. Many of the previously reported

![FIGURE 4](Image)

**FIGURE 4** HMOs reduce the ability of C. albicans to associate with pIECs. C. albicans SC5314 was incubated with pIECs for the indicated times. Values are means ± SEMs, n = 3. *Different from control, P < 0.05. HMO, human milk oligosaccharide; pIEC, premature intestinal epithelial cell.

![FIGURE 5](Image)

**FIGURE 5** HMOs reduce HSG expression by C. albicans. C. albicans SC5314 was incubated with pIECs and quantification of HSG expression was done by qPCR. Amount of HSG expression is presented as a percentage of control (ACT1) expression after 60 min of incubation. Combined expression (left-most bar) indicates the summed expression levels of 7 HSGs (HYR1, HWP1, ECE1, ALS3, HGC1, SAP5, and SAP8). Values are means ± SEMs, n = 3. *Different from control, P < 0.05. ACT1, yeast gene encoding actin 1; ALS3, yeast gene encoding agglutinin-like sequence 3; ECE1, yeast gene encoding extent of cell elongation 1; HGC1, yeast gene encoding hypha-specific expression and relatedness to G1 cyclins 1; HMO, human milk oligosaccharide; HSG, hyphal-specific gene; HWP1, yeast gene encoding hyphal wall protein 1; HYR1, hyphally regulated yeast gene 1; pIEC, premature intestinal epithelial cell; SAP5, yeast gene encoding secreted aspartyl proteinase 5; SAP8, yeast gene encoding secreted aspartyl proteinase 6.
beneficial effects of HMOs, with respect to preventing host–pathogen interactions in the intestine, appear to be mediated by specific HMO moieties rather than being generalizable to all HMO structures. Fucosylated oligosaccharides, in particular, have been shown to be the primary HMO component responsible for reducing the binding of C. jejuni to human intestinal tissue in vitro and mouse intestines in vivo (35), as well as for decreasing the diarrheagenic effects and mortality associated with Escherichia coli heat-stable enterotoxin in mice (10, 44, 45). Furthermore, for infants whose mothers have high concentrations of fucosylated oligosaccharides in their breastmilk, incidences of C. jejuni and calicivirus diarrhea are reduced compared with formula-fed infants (46). Of note, in studies examining the prevention of late-onset sepsis in infants, the use of a lactoferrin product containing fructo-oligosaccharides as a preservative was associated with a decrease in invasive Candida infections (47, 48), raising the possibility that oligosaccharides, lactoferrin, or a combination of the 2 compounds contributed to the reduction in fungal disease. In the current study, we used a pooled HMO preparation that was generated from multiple donors. Thus, the extent to which specific HMOs are contributing to reducing C. albicans interactions with pIECs remains unknown. Supplementation with specific commercially available oligosaccharides in infant formula or pumped maternal breast milk has not yet been proven to be effective for the prevention of infant diseases (4), and equivocal results have been observed for their effect on intestinal bacterial population structures (4, 49). It still remains possible that administration of an "optimized" HMO preparation, with respect to amounts and types of oligosaccharides, will prove to be an effective therapy in patients at risk of microbial-associated diseases that initiate in the intestine. Studies that aim to identify the specific oligosaccharide structures that are responsible for protecting pIECs from invasion and damage by C. albicans will be an important avenue for future research efforts.

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