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Synthetic Analogues of β-1,2 Oligomannosides Prevent Intestinal Colonization by the Pathogenic Yeast *Candida albicans*

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The pathogenic yeast *Candida albicans* displays at its cell surface β-1,2 oligomannosides (β-1,2-Mans). In contrast to the ubiquitous α-Mans, β-1,2-Mans bind to galectin-3, a major endogenous lectin expressed on epithelial cells. The specific role of β-1,2-Mans in colonization of the gut by *C. albicans* was assessed in a mouse model. A selected virulent strain of *C. albicans* (expressing more β-1,2-Man epitopes) induced more intense and sustained colonization than an avirulent strain (expressing less β-1,2-Man epitopes). Synthetic (Σ) β-and α-linked tetramannosides with antigenicities that mimicked the antigenicities of *C. albicans*-derived oligomannosides were then constructed. Oral administration of Σβ-1,2-Man (30 mg/kg of body weight) prior to inoculation with the virulent strain resulted in almost complete eradication of yeasts from stool samples, whereas administration of Σα-Man at the same dose did not. As most cases of human systemic candidiasis are endogenous in origin, this first demonstration that a synthetic analogue of a yeast adhesin can prevent yeast colonization in the gut opens the possibility of new prophylactic strategies.

Since the 1980s the incidence of systemic *Candida* infections in hospitalized patients has increased dramatically, and yeasts of the genus *Candida* are now the fourth most important cause of nosocomial infection (1a, 35). Patients at risk of developing systemic candidosis are immunosuppressed as a result of their primary illness and/or the medicosurgical procedures designed to control or cure it. These patients are usually hospitalized on highly specialized wards (oncology wards, hematology wards, intensive care, neonatal units, and surgical wards, including organ transplantation units). The medical and economic problems associated with these opportunistic infections result from the difficulties in establishing a rapid and specific diagnosis and have led to considerable investment in both basic academic research and antifungal drug development.

Candida albicans, the most pathogenic *Candida* species, is responsible for 60 to 80% of infections and is a harmless commensal of the digestive tract of at least 50% of healthy subjects (31). The percentage of colonized subjects and the intensity of gut colonization both increase as a result of perturbation of host homeostasis during hospitalization (31). In both intensive care units (33) and clinical hematology units, colonization by *C. albicans* has been shown to be an independent risk factor for the development of systemic candidosis (26). In addition, genetic similarities have been found between strains colonizing patients and those recovered from blood cultures (29, 36, 50). Thus, the prevention or reduction of yeast adherence in the gut could have prophylactic effects. Several studies have established the role of ligand-receptor interactions in colonization of different segments of the host digestive tract by *C. albicans*. Although mannose residues make up 40% of the cell wall (25) and/or play a part in the relationship between the cell surface of *C. albicans* and its environment (6), few studies have investigated the role of mannose residues in this interaction. Among them, a role for *C. albicans* antigen 6, specific for serotype A, has been demonstrated in the adherence to buccal epithelial cells (30). In nonpathogenic yeasts such as *Saccharomyces cerevisiae* and in mannoconjugates formed by enzymes encoded by viruses, bacteria, and parasites, most mannose sequences correspond to mannose residues linked through α-1,6, α-1,2, or α-1,3 bonds. *C. albicans* contains enzymes capable of constructing unique sequences of mannose residues linked through an unusual anomer type of linkage, β-1,2 oligomannosides (β-1,2-Mans) (42). These residues are expressed in large quantities at the cell wall surface in association with different molecules, either mannan (42), mannoproteins (47, 48), or glycolipids (49). β-1,2-Mans act as adhesins (14, 28) and trigger macrophages producing different mediators modulating the host response (23, 24), while β-1,2-Mans at the nonreducing end of α-linked chains have been shown to act as adhesins for buccal epithelial cells (30). β-1,2-Mans also induce specific antibodies, which, in contrast to antibodies directed against α-linked mannose residues, protect animals from either systemic candidosis (18) or vaginal candidosis (19). Furthermore, a previous study in our laboratory demonstrated that recognition of β-1,2-Mans by endogenous lectins of mammals occurred through galectin-3 and did not involve C-lectins, which bind to mannose residues with an α-anomer type of linkage (15). Galectin-3 is a major lectin with pleiotropic effects expressed on a large variety of cells includ-

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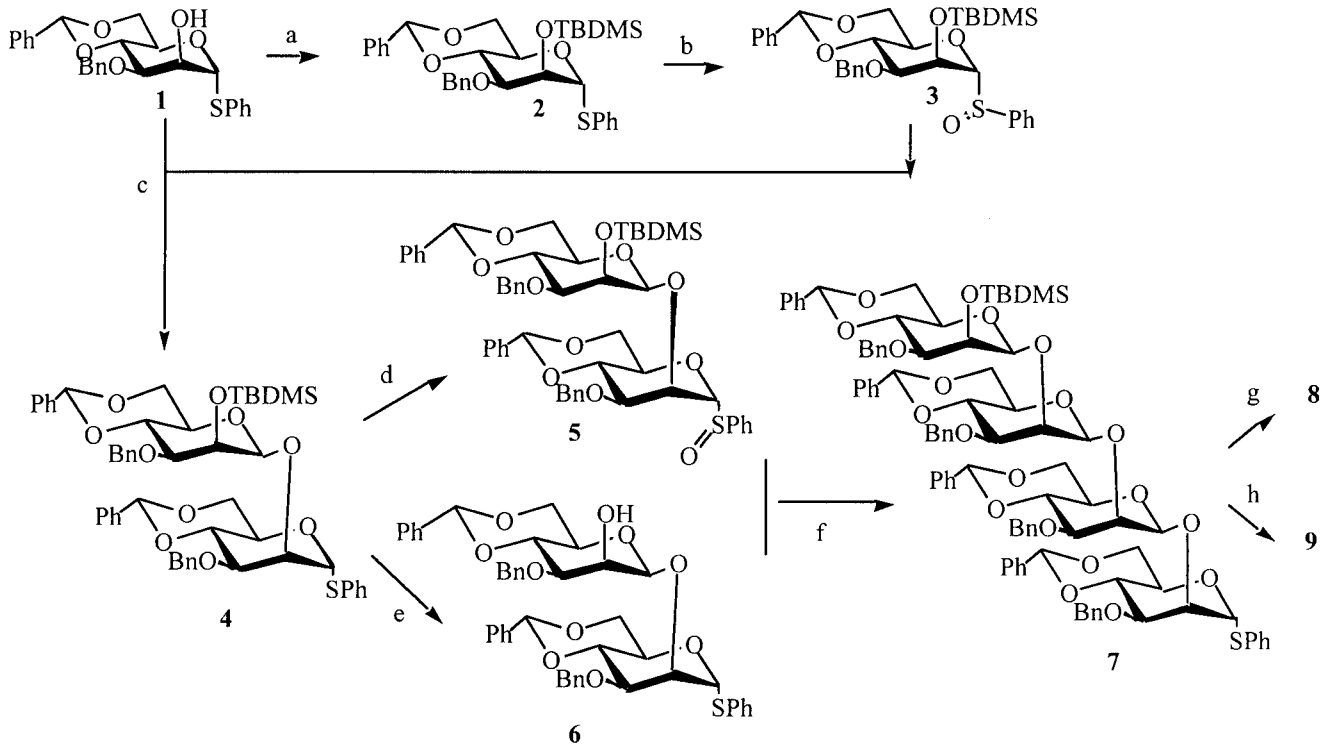

FIG. 1. Preparation of β-mannosides, reagents, and conditions (numbers in boldface refer to the various compounds). (a) Protection of OH-2 by a tertbutyldimethylsilyl group: tertbutyldimethylsilyl triflate, triethylamine, CH_2Cl_2, 20°C, 15 h, 90%. (b) Oxidation of the phenylsulfide into a phenylsulfoxide: *meta*-chloroperbenzoic acid, CH_2Cl_2, 79%. (c) Glycosylation by activation of the anomeric phenylsulfoxide: triflic anhydride, 2,6-ditertbutyl-4-methylpyridine, −78°C, CH_2Cl_2, 72%. (d) Oxidation of the phenylsulfide into a phenylsulfoxide: *meta*-chloroperbenzoic acid, CH_2Cl_2, 91%. (e) Removal of the tertbutyldimethylsilyl group at OH-2: $N(n\text{-}C_4H_9)_4F$, aqueous tetrahydrofuran, 20°C, 1 h, 90%. (f) Glycosylation by activation of the anomeric phenylsulfoxide: triflic anhydride, 2,6-ditertbutyl-4-methylpyridine, CH_2Cl_2, −78°C, 55%. (g) Deprotection (three steps): first step (removal of the tertbutyldimethylsilyl group at OH-2), $N(n\text{-}C_4H_9)_4F$, aqueous tetrahydrofuran, 60°C, 12 h, 92%; second step (hydrolysis of the thiophenyl group), *N*-bromosuccinimide, acetone-H_2O, 0°C, 30 min, 84%; third step (removal of the benzyl ethers and benzylidene groups), H_2, Pd-C, methanol, 85%. (h) Glycosylation of the linker and deprotection (four steps): first step (introduction of the linker), 8-methoxycarbonyloctanol, *N*-bromosuccinimide, triflic acid, 4-Å molecular sieves, CH_2Cl_2, −20°C, 1 h, 70% β/α = 6/1; second step (removal of the tertbutyldimethylsilyl group at OH-2), $N(n\text{-}C_4H_9)_4F$, aqueous tetrahydrofuran, 60°C, 12 h, 80%; third step (saponification of the ester group), NaOH, aqueous tetrahydrofuran, 82%; fourth step (removal of the benzyl ethers and benzylidene groups), H_2, Pd-C, methanol, 83%. Abbreviations: Ph, phenyl; Bn, benzyl; TBDMS, tertbutyldimethylsilyl.

ing intestinal epithelial cells (9). This suggests that β-1,2-Mans could have a specific role in the interaction between *C. albicans* and its natural ecological niche.

An interdisciplinary approach was therefore designed to determine the role of β-1,2-Mans in gut colonization in comparison to those of the α-linked mannose residues expressed by other commensal organisms or pathogens of the gut. In a preliminary study, the virulence of a number of *C. albicans* strains was demonstrated to correlate closely with the level of β-1,2-Man epitope surface expression. Synthetic β- and α-1,2 mannotetraoses were then constructed, and their antigenicities were shown to mimic the antigenicities of native *C. albicans* homologues by using polyclonal and monoclonal antibodies (MAbs) specific for the native epitopes. By using the highly standardized infant mouse model developed by Cole et al. (7), administration of synthetic (Σ) β-1,2-Mans (Σβ-Mans) prior to inoculation with *C. albicans* was shown to prevent gut colonization by a virulent strain, whereas synthetic α-Mans (Σα-Mans) had no effect.

MATERIALS AND METHODS

***C. albicans* strains.** The *C. albicans* strains used in this study were kindly provided by A. Schmidt (Bayer AG, Wuppertal, Germany), who has previously shown that these strains are reproducibly distributed into three groups according to their virulence in mouse and rat models of *C. albicans* systemic infection. From this panel of strains, we selected only the seven serotype A strains. They are classified as avirulent (strains ATCC 44831, ATCC 18804, and ATCC 10231), virulent (ATCC 44505, ATCC 62342, and ATCC 10261), and intermediate (ATCC 32354). The strains were maintained as stock suspensions at −80°C in 40% glycerol. The yeasts were always used after culture on Sabouraud's dextrose agar at 37°C and three washes in sterile saline.

Polyclonal antibodies and MAbs against *C. albicans* oligomannose residues. Monospecific rabbit antisera (Candida check; Iatron Laboratories Inc., Tokyo, Japan) were used. Specifically, for the present study, serum factor 1, which recognizes α-1,2 mannose sequences [Man (α-1,2-Man)$_n$ (α-1,2-Mans with $n \geq 0$)], and serum factor 5, which reacts with a β-1,2 oligomannose sequence [Man (β-1,2-Man)$_n$ (β-1,2-Mans with $n \geq 1$)] (45), were used.

MAb AF1 from Cassone et al. (5) and MAb DF9-3 from Borg-Von Zepelin and Gruness (2) were also used. These MAbs react specifically with β-1,2-Mans (48, 49). MAb EBCA1 was used as a control for α-linked mannose (22). This antibody is used in a commercially available test (Platelia *Candida*; Bio-Rad, Marnes-la-Coquette, France) for the detection of mannanemia (41).

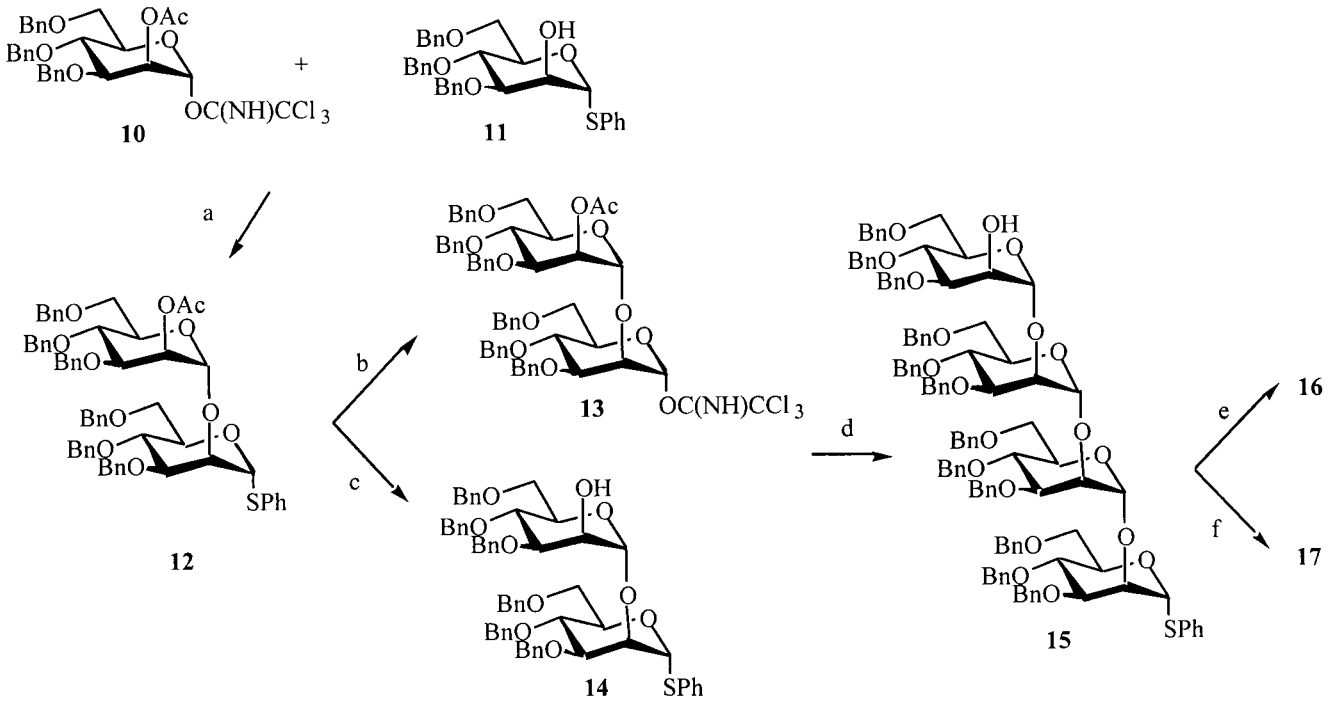

FIG. 2. Preparation of α-mannosides, reagents, and conditions (numbers in boldface refer to the various compounds). (a) Glycosylation by activation of the trichloroacetimidate: trimethylsilyl triflate, 4-Å molecular sieves, CH_2Cl_2, −10°C, 30 min, 72%. (b) Preparation of the disaccharide donor (two steps): first step (hydrolysis of the anomeric phenyl sulfide), *N*-bromosuccinimide, acetone-H_2O, 20°C, 15 min, 87%; second step (formation of the anomeric trichloroacetimidate), CCl_3CN, 1,8-diazabicyclo(5,4,0)undec-7-ene, CH_2Cl_2, 0°C, 20 min, 81%. (c) Preparation of the disaccharidic acceptor: Na, methanol, toluene, 20°C, 15 min, 95%. (d) Glycosylation by activation of the trichloroacetimidate: $BF_3(C_2H_5)2O$, 4-Å-mesh-size molecular sieves, CH_2Cl_2, −10°C, 40 min, 60%, and then removal of the acetate group: Na, toluene, methanol, 20°C, 15 min, 71%. (e) Deprotection (two steps): first step (hydrolysis of the anomeric phenyl sulfide), *N*-bromosuccinimide, acetone-H_2O, 0°C, 30 min, 84%; second step (removal of the benzyl ethers), H_2, Pd-C, methanol, 85%. (f) Glycosylation of the linker and deprotection (three steps): first step (introduction of the linker), 8-methoxycarbonyloctanol, *N*-bromosuccinimide, triflic acid, 4-Å molecular sieves, CH_2Cl_2, −20°C, 1 h, 72% β/α = 1/1; second step (saponification of the ester group), NaOH, aqueous tetrahydrofuran, 78%; third step (removal of the benzyl ethers), H_2, Pd-C, methanol, 95%. Abbreviations: Ph, phenyl; Bn, benzyl.

Chemical synthesis of *C. albicans* oligomannose analogues. The chemical reactions used to synthesize the *C. albicans* oligomannose analogues are shown schematically in Fig. 1 for Σβ-Mans and in Fig. 2 for Σα-Mans. The Σβ-Mans D-Man*p* $\beta(1\rightarrow2)_4$ (compound 8) and D-Man*p* $\beta(1\rightarrow2)_4$ O-$(CH_2)_8$-COOH (compound 9) were synthesized from compound 1 (Fig. 1), prepared from D-mannose (D-Man) by previously published methods (32, 46) by using the sulfoxide strategy recently applied to the construction of β-manno linkages by Crich and Sun (10). This glycosylation method is compatible with the presence of a thiophenyl group in the acceptor and thus allows convergent blockwise synthesis of the tetrasaccharide (compound 7). This pivotal compound was either deprotected to give compound 8 or coupled with 8-methoxycarbonyloctanol, prepared from azelaic acid by the method of Lemieux et al. (27), to give compound 9 after deprotection.

The synthesis of the α-mannosides (46) D-Man*p* $\alpha(1\rightarrow2)_4$ (compound 16; Fig. 2) and D-Man*p* $\alpha(1\rightarrow2)_4$ O-$(CH_2)_8$-COOH (compound 17) was carried out starting from compounds 10 and 11, respectively, prepared from D-Man by previously published methods (51, 53). A blockwise synthesis was also used, the thiophenyl group of compound 14 being stable under trichloroacetimidate (13) activation conditions. The tetramannoside (compound 15) was converted into compounds16 and 17 in a manner analogous to that described for glycosylation and deprotection of compound 7.

Assessment of synthetic oligomannose antigenicities against MAbs by EIA. The oligosaccharides 9 and 17 were first coupled to CH_2-$(CH_2)_7$-COOH (linker developed by Lemieux et al. [27]) through their reducing terminal ends. The connecting residue was then covalently linked to the wells of a microtiter plate (CovaLink NH_2; Nunc, Roskilde, Denmark) by using carbodiimide (43) to provide serial concentrations. The plates were rinsed three times with phosphate-buffered saline (PBS; 200 μl/well) and blocked with PBS plus 5% nonfat dry milk overnight at 4°C (200 μl/well). The plates were washed five times with 200 μl of TNT (50 mM Tris-HCl, 150 mM NaCl, 0.05% Tween 20 [pH 7.5]) per well. Enzyme immunoassay (EIA) was then performed as described previously (12, 41).

Evaluation of β-1,2-Man surface expression by latex agglutination. Carboxyl-modified microspheres (Estapore K1-0.8) were obtained from Prolabo (Fontenay-sous-Bois, France) and coated with purified MAbs AF1 and DF9-3 as described previously (39) by a procedure used in the Bichro-latex test developed by one of us. Sensitized red particles were then suspended in a green buffer designed to prevent direct yeast agglutination. Before use, the reagent was homogenized to form a dark brown suspension.

A suspension of 10^6 yeasts/ml was prepared from fresh cultures for each experiment. The coagglutination test was performed by mixing 15 μl of yeast suspension (10^6 yeasts/ml) with 15 μl of sensitized particles, followed by rotation of the mixture. Preliminary experiments showed that a scoring procedure ensured the reproducibility of the results. Thus, the absence of reactivity producing a homogeneous dark brown spot due to nonagglutinated red particles evenly suspended in the green buffer was assigned a score of 0, while positive results were graded 0.5 to 2 as follows: reactions in which all the agglutinated beads formed a thick, red edge around a clear, green central area were given a score of 2; reactions with weaker agglutination, a thinner colored edge, and some red agglutinates remaining in the central part of the spot in contrast to the green background were given a score of score 1; very weak reactions that gave only small red aggregates without any visible edge were given a score of score 0.5. Agglutination scores were recorded after 1, 2, and 3 min; their sums gave the score for each MAb; and these scores were added to give a final score, which thus took into account the speed and intensity of the agglutination reaction. The tests were performed blindly three times with each of the strains provided by A. Schmidt, and the results were subsequently deciphered.

Experimental model of gastrointestinal colonization. The experimental model of gastrointestinal colonization was based on that described by Pope et al. (34).

Suckling Swiss mice (age, 3 to 4 days; CD1, Crl:CD1, BR mice; ICR) were obtained from Charles River Laboratories (St. Aubin les Elbeuf, France) as litters of 10 to 15 animals. Six-day-old infant mice weighing approximately 5 g were removed from their mothers for 3 h, inoculated, and placed back with their dams 1 h after gavage.

Two strains (ATCC 10241 and ATCC 10261) were selected for determination of differences in their levels of surface β-1,2-Man expression (see Results). The yeast suspensions were adjusted to 2×10^9/ml in sterile water, and viability was determined by counting the numbers of CFU in duplicate. The suspension was administered by gavage in a 50-μl volume with a 1-ml syringe equipped with the Teflon tubing of a sterile intravenous catheter (Insyte 24 GA, 0.7 by 19 mm; Becton Dickinson and Co., Paramus, N.J.).

The course of the infection was monitored daily by counting the number of surviving infant mice in each cage. The degree of gut colonization was assessed by measuring the numbers of CFU in the fecal pellets (8). Fresh fecal pellets were collected from each mouse and immediately homogenized in 100 μl of sterile distilled water, and the suspension obtained was plated onto Sabouraud's dextrose agar containing 50 μg of chloramphenicol per ml. Since the small size of the fecal pellets prevented accurate weighing, the first dropping was always used for the experiments. After incubation at 30°C for 24 h, the number of CFU was counted. When the CFU counts were >300 to 400, a quick evaluation was performed on a small portion of the plate and an arbitrary number was used to allow comparison between groups: 1,000 for colonies that were still distinct, 10,000 for nonconfluent growth, and 100,000 for confluent growth. It must be noted that for the noninfected animals the first fecal pellets obtained from infant mice (on day 13 after birth) and those from adults were consistently negative (data not shown).

The synthetic oligosaccharides (Σβ-Mans and Σα-Mans) were dissolved in sterile distilled water to a concentration of 3 or 1 mg/ml. The solution was administered by gavage in a 50-μl volume 1 h prior to oral inoculation. The effects of pretreatment with water (litter 1), Σβ-Mans at 50 μg/infant mouse (10 mg/kg of body weight; litter 2), Σβ-Mans at 150 μg/infant mouse (30 mg/kg; litter 3), or Σα-Mans at 150 μg/infant mouse (litter 4) on gut colonization were evaluated.

Statistical analysis. The percent mortality and the percentage of animals with positive feces were compared between litters infected with strains ATCC 10231 and ATCC 10261 by the Fisher exact test. Nonparametric tests (the Mann-Whitney or the Kruskal-Wallis test, depending on the number of groups) were used for comparison of gut colonization and agglutination scores.

RESULTS

Synthetic oligomannose residues with antigenicities that mimic the antigenicities of natural components of the *C. albicans* cell wall can be constructed. Figure 3 shows the reactivities of serum factors 1 and 5, specific for α-1,2 oligomannose and β-1,2 oligomannose sequences, respectively, with Σα-Mans and Σβ-Mans coupled to EIA plates. Increasing concentrations of both Σβ-Mans and Σα-Mans were associated with a dose-dependent binding of serum factors specific for each epitope, whereas only weak reactivity was observed with the irrelevant epitope. Figure 4 shows the reactivity of MAb AF1 (specific for β-linked oligosaccharides) and MAb CA1 (specific for α-linked oligomannosides) with Σβ-Mans. Poor binding of MAb CA1 was observed, whereas the binding of MAb AF1 increased in parallel with the amount of Σβ-Mans coated onto the plates. Thus, the antigenicities of the synthetic oligomannosides appeared to mimic closely the antigenicities of native *C. albicans* molecules.

Surface expression of β-1,2-Man epitopes correlates with virulence of *C. albicans* serotype A strains in systemic models of candidosis in rats and mice. Seven nonisogenic strains previously shown (40) to exhibit reproducible differences in virulence in rat and mouse models of systemic infection were tested blindly for their abilities to react with MAbs specific for β-1,2-Mans. The level of epitope expression was measured by determination of both the sizes of the agglutinates and the times required for the reaction to develop, with the highest agglutination score relating to the highest level of surface expression of β-1,2-Mans.

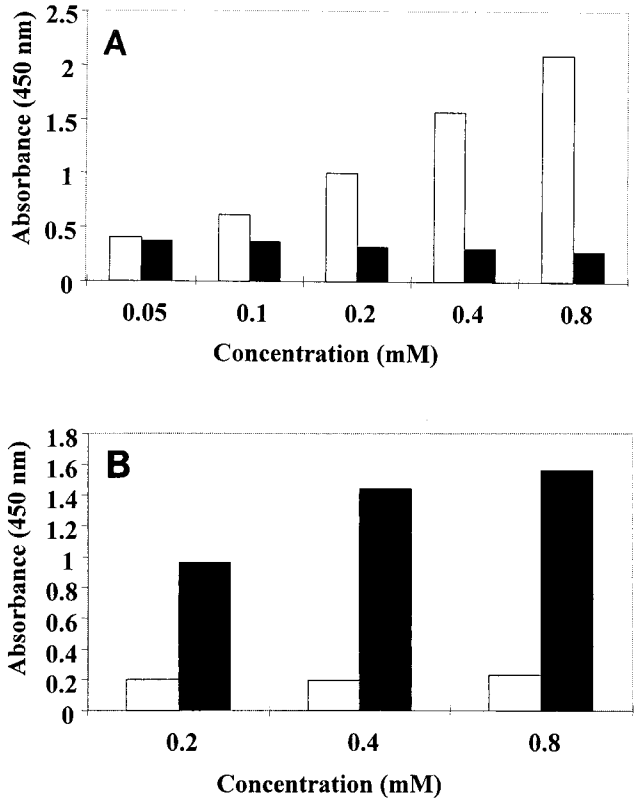

FIG. 3. Binding of polyclonal factor sera to synthetic α- and β-mannotetraoses as measured by EIA. Serum factor 5 (reactive with β-1,2 mannose sequence; closed bars) and serum factor 1 (reactive with α-1,2 mannose sequence; open bars) were allowed to react with plates coated with increasing concentrations of synthetic α-1,2-linked mannotetraose (A) and β-1,2-linked mannotetraose (B).

As shown in Table 1, the agglutination scores were significantly lower for strains with low levels of virulence than for those with high or intermediate levels of virulence (medians, 5.5 and 7.7, respectively [$P = 0.034$]), suggesting a correlation between the surface expression of β-1,2-Mans and virulence.

Virulence of two *C. albicans* strains in the infant mouse model of gut colonization correlates with virulence in systemic models and surface expression of β-1,2-Mans. One virulent strain (ATCC 10261) with a high level of β-1,2-Man surface expression and one avirulent strain (ATCC 10231) with a low level of β-1,2-Man surface expression were selected. Their virulences were compared in six independent experiments. Preliminary analysis showed that the degree of gut colonization evaluated according to the number of CFU per fecal pellet was reproducible, allowing the data for two litters infected with the same strain to be pooled (data not shown). Results from representative experiments are shown in Table 2. The results are for three litters inoculated with each strain, with the results for a total of 31 animals infected with strain ATCC 10261, 30 animals infected with strain ATCC 10231, and 217 stool cultures provided.

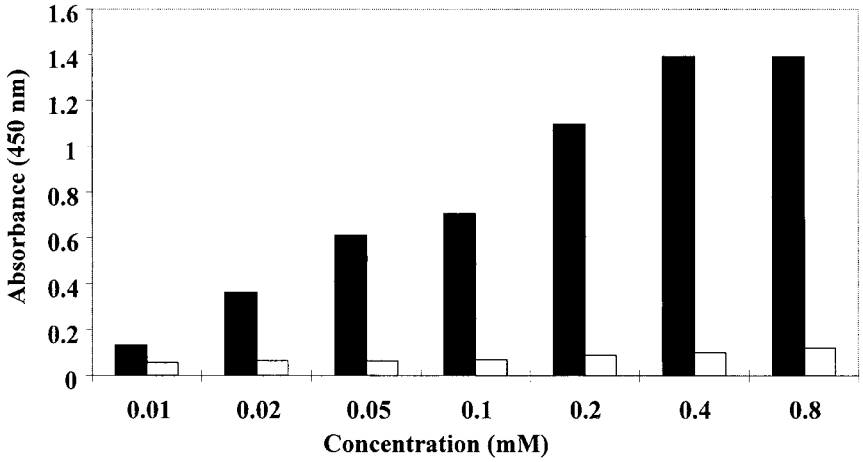

FIG. 4. Binding of anti-*C. albicans* MAbs to synthetic β-1,2-linked mannotetraose measured by EIA. MAb AF1, which is specific for *C. albicans* β-1,2-Mans (closed bars), and MAb CA1, which reacts with *C. albicans* α-linked mannose residues (open bars), were incubated with increasing concentrations of synthetic β-1,2-linked mannotetraose.

From day 7 to day 33 after inoculation (day 7 was the first day on which it was possible to assess colonization), colonization was variable from mouse to mouse but was always significantly higher in mice colonized with strain ATCC 10261 than in those colonized with strain ATCC 10231. Thus, colonization was more protracted in animals infected with ATCC 10261 than in those infected with ATCC 10231 (at day 33 postinoculation, 11 of 11 animals inoculated with ATCC 10261 were still colonized, whereas 5 of 11 animals infected with ATCC 10231 were still colonized [$P = 0.006$]). The rate of mortality for animals without subsequent induced immunosuppression was low. However, the rate of cumulative acute mortality from the six independent experiments was higher among infant mice inoculated with ATCC 10261 than among infant mice inoculated with ATCC 10231, although the difference did not reach statistical significance (6 of 63 versus 1 of 62 mice [$P = 0.059$]).

Synthetic β-1,2 tetramannosides but not α-1,2 tetramannosides inhibit gut colonization by a virulent *C. albicans* strain.

TABLE 1. Agglutination scores for *C. albicans* strains reacted with Bichro-latex particles sensitized with MAbs DF9-3 and AF1 specific for β-1,2-Man epitopes compared with virulence determined from systemic *Candida* infection of rats and mice[a]

Strain	Cumulated score obtained with beads sensitized with MAb:			Virulence observed in vivo
	DF9-3	AF1	Final score	
ATCC 44505	4	2.5	6.5	Virulent
ATCC 62342	4.5	4	8.5	Virulent
ATCC 10261	4.5	4.5	9	Virulent
ATCC 32354	4.5	2.5	7	Intermediate
ATCC 44831	2.5	3	5.5	Avirulent
ATCC 18804	3	2.5	5.5	Avirulent
ATCC 10231	3.5	2	5.5	Avirulent

[a] The agglutination scores (obtained at 1, 2, and 3 min with the same suspension) were determined, and the final score (addition of the scores obtained with MAbs DF9-3 and AF1) was used for statistical comparison (results of a typical experiment). The virulences of the strains were determined as described previously (40).

The last set of experiments was designed to test the prophylactic effects of β-1,2-Mans. Synthetic tetramannosides were administered as a single dose prior to *C. albicans* inoculation. In a preliminary experiment, the effect of the administration of 50 μg of Σβ-Mans prior to inoculation of *C. albicans* strains ATCC 10261 and ATCC 10231 was evaluated at day 7 postinoculation. Pretreatment had no effect when the mice were inoculated with the strain expressing less β-1,2-Mans at the cell surface (ATCC 10231), while there was a reduction (although it was not significant) in the numbers of CFU from mice inoculated with the strain expressing more β-1,2-Mans (median counts, 0 CFU [range, 0 to 36 CFU] versus 1 CFU [range, 0 to 9 CFU] and 71 CFU [range, 4 to 482 CFU] versus 14 CFU [range, 0 to 171 CFU] for strains ATCC 10231 and ATCC 10261, respectively). To further study the specificity of the effect, we decided to use the more virulent strain expressing more β-1,2-Mans, larger numbers of animals, different doses of Σβ-Mans, and Σα-Mans as a control (Fig. 5). At day 7 after inoculation, administration of Σα-Mans had no effect on gut colonization compared to the effects of distilled water (control treatment) (median counts, 196 CFU [range, 11 to 1,000 CFU] versus 258 CFU [range, 31 to 1,000 CFU] in the Σα-Man-treated group versus the controls [$P > 0.05$]). By contrast, administration of 50 μg of Σβ-Mans led to a decrease in the number of CFU per fecal pellet, which was significant. The effect of Σβ-Mans was dose dependent, since the effect became highly significant when 150 μg was administered. The efficacy of this treatment was further demonstrated by the complete disappearance after treatment with Σβ-Mans of the variability in the level of gut colonization observed in all experiments among untreated infant mice and in this experiment among untreated and Σα-Man-treated animals (Fig. 5 and Table 2). We also sampled the animals on day 10 after inoculation and observed that the level of colonization had dramatically increased in all mice, but it had not increased as much in mice treated with 150 μg of Σβ-Mans than in the other groups ($P = 0.0220$).

TABLE 2. Kinetics of gut colonization in infant mice after oral inoculation with *C. albicans* strains ATCC 10231 and ATCC 10261 expressing low and high levels of β-1,2-Mans, respectively, at their cell surfaces

Day after inoculation	Median (range) no. of CFU/fecal pellet[a] (no. of expts)		Significance[b]
	ATCC 10231	ATCC 10261	
Expt A			
7	0.5 (0–31) (n = 10)	64.5 (0–1,000) (n = 12)	0.0011
8	2 (0–40) (n = 11)	1,000 (35–1,000) (n = 13)	0.0001
9	64.5 (0–475) (n = 10)	256 (160–10,000) (n = 13)	0.0001
10	510 (8–1,000) (n = 10)	1,000 (300–10,000) (n = 12)	0.0001
Expt B[c]			
12	107 (8–500) (n = 19)	548 (144–1,000) (n = 18)	<0.0001
15	135 (25–1,000) (n = 12)	10,000 (500–10,000) (n = 11)	0.0003
19	17 (4–167) (n = 11)	1,000 (196–1,000) (n = 11)	<0.0001
26	7.5 (0–82) (n = 11)	398 (28–1,000) (n = 11)	0.0006
33	0 (0–9) (n = 11)	484 (7–10,000) (n = 11)	<0.0001

[a] CFU were enumerated, and an arbitrary number (see the text) was used for counts >300 to allow statistical comparison by nonparametric tests.
[b] P value for comparison of the number of CFU per fecal pellet in ATCC 10231-infected mice versus the number in Mann-Whitney test. ATCC 10261-infected mice on the same day. Significance was determined by the Mann-Whitney test.
[c] Results for 2 litters were pooled after the reproducibilities of the results between litters infected with the same isolate were checked (data not shown). The groups were each composed of 5 to 10 animals, depending on the day of study.

DISCUSSION

Although *C. albicans* is not a true pathogen, it has some biological characteristics, termed virulence factors (3), which help it to invade debilitated hosts. Among these factors are cell wall molecules that act as adhesins (44). *C. albicans* has been shown to adhere to a wide variety of host cells and molecules through protein-protein (17), yeast lectin-host carbohydrate (4, 52), or yeast carbohydrate-host lectin interactions. A growing body of evidence suggests that β-1,2-Mans have an important role in pathogenesis since they have been shown to act as adhesins (28), stimulators of immune response mediator secretion (23, 24), and inducers of protective antibodies (18, 19). The recent identification of galectin-3 (previously, the Mac 2 antigen) as a β-1,2-Man receptor on host cells provides further evidence for the specificity of these interactions (15). It indeed appears that *C. albicans* has a unique biological trait related to its ability to express β-1,2-Mans at its cell surface and that this biological trait has its counterpart in two major host recognition systems, namely, antibodies and endogenous lectins. It therefore seemed worthwhile to explore the role of this system

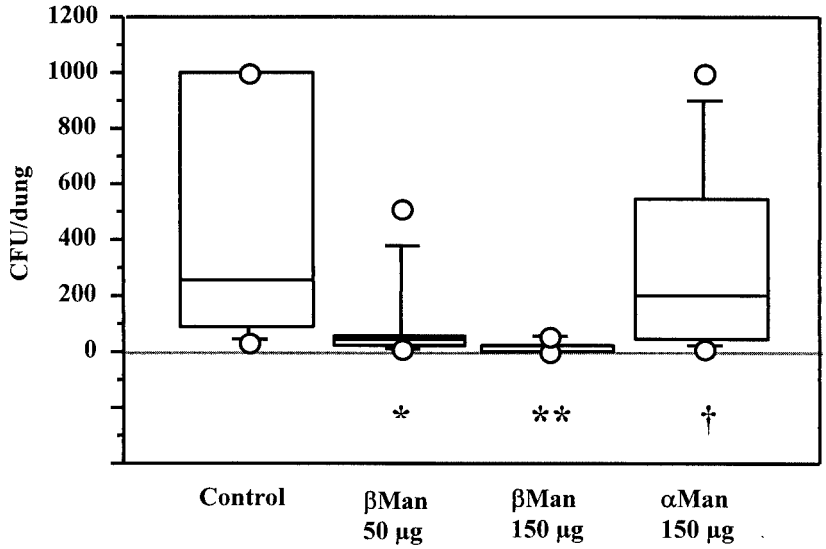

FIG. 5. Prophylactic effects of synthetic mannotetraoses on gut colonization of infant mice by *C. albicans* ATCC 10261, a strain that expresses high levels of β-1,2 mannose at its cell surface. Infant mice received either distilled water (control) or solutions providing 50 μg of synthetic β-1,2 tetramannose (β-Man), 150 μg of β-1,2 tetramannose, or 150 μg of synthetic α-1,2 tetramannose (α-Man) per mouse 1 h before being inoculated with ATCC 10261. The degree of gut colonization was evaluated at day 7 after inoculation by counting the number of CFU per fecal pellet (9 to 11 mice per group). Data are shown as boxes, in which the internal horizontal lines indicate medians; the tops and bottoms of the boxes represent the 25th and 75th percentiles, respectively; the upper and lower bars represent the 10th and 90th percentiles, respectively; and open circles represent values exceeding the range of 10 to 90%. *, $P < 0.02$ versus 50 μg of β-1,2 tetramannose; **, $P < 0.0001$ versus 150 μg of β-1,2 tetramannose; †, not significant versus 150 μg of α-1,2 tetramannose (P values were determined by the Kruskal-Wallis test).

in the process of colonization of the gut, where galectin-3 is widely expressed (9).

To address this question of the role of β-1,2-Mans in colonization, a multiple-step study was designed. Previous data suggested a relationship between the expression of β-1,2-Mans at the yeast cell surface and the virulence of strains (16). By testing several unrelated strains of *C. albicans* that have been shown to exhibit various degrees of virulence in animal models of systemic infection (40), a clear correlation was found between these two parameters; namely, the weaker the expression of β-1,2-Mans is, the lower the reported level of virulence is, and vice versa. This is the first set of experiments suggesting a correlation between surface expression of β-1,2-Mans and *C. albicans* strain pathogenicity in animal models. The same trend was observed when more than 200 *C. albicans* clinical isolates were tested in a previous study (16). The isolates recovered from colonized patients were less agglutinated by an MAb designated 5B2 than those isolated from infected patients. Interestingly, subsequent immunochemical experiments showed that MAb 5B2 is specific for β-1,2-Man epitopes (48). In the present study the strain with the higher levels of expression of β-1,2-Man epitopes was shown to induce higher levels of colonization and more protracted colonization of the gut of infected mice than the strain with lower levels of expression of β-1,2-Man epitopes.

To further assess the role of β-1,2-Mans in intestinal colonization, synthetic oligosaccharides were administered as potential competitors prior to inoculation with the most virulent strain. Preparation of large quantities of mannan-derived oligomannosides is based on a long and tedious procedure and requires nuclear magnetic resonance verification of the structures obtained (12). As previous studies with macrophages demonstrated that synthetic molecules exhibiting a minimum polymerization of four residues inhibit β-1,2-Mans binding and stimulation of mammalian cells (14, 24), it was decided to chemically synthesize β-1,2 mannotetraoses. Appropriate controls, consisting of α-1,2-Man tetramannoses, were also synthesized. EIA demonstrated that the antigenicities of both Σα-Mans and Σβ-Mans mimicked the antigenicities of *C. albicans*-derived homologues. When animals were given Σβ-Mans (30 mg/kg) prior to inoculation with *C. albicans*, a dramatic decrease in the number of yeasts recovered from stools was observed; however, this decrease was not seen following administration of the same dose of Σα-Mans. To us, this effect is of real biological significance because it was evidenced 7 days and even 10 days after administration. Furthermore, it was dose and structure dependent.

Together these data strongly suggest that expression of β-1,2-Mans at the cell surface of *C. albicans* has an important role in gut colonization. In hospitalized immunocompromised patients, it is now agreed that sustained gastrointestinal colonization usually precedes disseminated infection (33). Prophylaxis with nonabsorbable oral antifungal agents has been advocated to reduce gastrointestinal colonization, but the efficacy of this approach for decontamination of the digestive tract has not been demonstrated conclusively (11, 20). Azole antifungal agents have been more successful at reducing colonization and infection, but their intensive and/or inappropriate use may select for resistant strains or species (11, 37). Another means of controlling the proliferation of one species is to change the indigenous flora by providing a local competitor. When infant mice were inoculated with two different *Candida* species, the number of mice colonized with *Candida glabrata* increased as the number of mice colonized with *C. albicans* decreased (13). The *C. albicans* competitor used in human studies is the nonpathogenic yeast *Saccharomyces boulardii* (21), but conflicting results and rare cases of fungemia due to *Saccharomyces* species may discourage use of this approach (38).

Even if the efficacy of Σβ-Mans is transient (since we chose to administer only a single dose), our data suggest that this innovative approach may be useful for the prevention of systemic *Candida* infection in at-risk patients. This strategy has several potential advantages over existing prophylactic regimens: (i) the chemical procedure can be easily adapted to the production of large quantities of β-Mans; (ii) Σβ-Mans are resistant to gastric pH and should also be well tolerated, as β-1,2-Mans (from *C. albicans*) are already present in the human gut; (iii) Σβ-Mans should not generate resistance; and (iv) the effective dose (30 mg/kg) that leads to a lasting effect is relatively low, at least in mice. Further studies with different animal models are in progress to determine the influence of the administration regimen (timing, doses) on the therapeutic efficacies of Σβ-Mans for the long-lasting prevention of gut colonization and to determine whether it extends to a reduction in the level of existing colonization or the treatment of existing colonization. In vitro studies are also in progress since, besides their therapeutic applications, the clear-cut results obtained here are of basic pathophysiological and phylogenetic interest for the understanding of host-endogenous microbe interactions.

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