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Lactobacillus johnsonii La1 shares carbohydrate-binding specificities with several enteropathogenic bacteria

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The carbohydrate-binding specificities of the probiotic lactic acid bacterium *Lactobacillus johnsonii* La1 (a health-beneficial bacterial strain able to be incorporated into the human intestinal microflora) were investigated *in vitro*. First various soluble complex carbohydrates were tested as potential inhibitors of the strain adhesion onto Caco-2 intestinal epithelial cells, and then bacterial binding to glycolipids immobilized on TLC plates was probed. Two major carbohydrate-binding specificities of *Lactobacillus johnsonii* La1 were identified. A first one for an Endo-H treated yeast cell wall mannoprotein carrying mainly *O*-linked oligomannosides, and a second one for the gangliotri- and gangliotetra-osylceramides (asialo-GM1). Similar carbohydrate-binding specificities are known to be expressed on cell surface adhesins of several enteropathogens, enabling them to adhere to the host gut mucosa. These findings corroborate the hypothesis that selected probiotic bacterial strains could be able to compete with enteropathogens for the same carbohydrate receptors in the gut.

Key words: glycolipids/mannoprotein/intestinal epithelial cells/ *Lactobacillus johnsonii* La1/probiotic bacterial adhesion

Introduction

It is more and more recognized that the resident microflora of the gastrointestinal tract plays an important role in inhibiting gut colonization by incoming pathogens (Tancrede, 1992; Berg, 1996). Different mechanisms enable this normal flora to play such a role (Finlay and Siebers, 1995): first, some strains or species can prevent pathogen adherence by specifically competing for the same receptor sites, or alternatively by providing an aspecific steric hindrance as a barrier to mucosal colonization; second, the production of antimicrobial metabolites (bacteriocinlike or nonbacteriocin substances) by the normal flora can remove pathogens from the intestine; and third, some strains can exhibit an enhancement of the host immune response against pathogens. Recently, the idea has emerged to select among lactic acid bacterial strains those which are able to be incorporated into the resident flora and to demonstrate beneficial potentialities, then to investigate their biological effects both *in vitro* and *in vivo*, and finally use them in dairy products offering health benefits (Lee and Salminen, 1995; Brassart and Schiffrin, 1997). One of these "probiotic" (Brassart and Schiffrin, 1997) bacterial strains, *Lactobacillus johnsonii* La1 (formerly *Lactobacillus acidophilus* La1), has shown immunomodulatory properties on the one hand (Link-Amster *et al.*, 1994; Schiffrin *et al.*, 1995; Haller *et al.*, 2000), and antipathogenic properties on the other hand, both *in vitro* and *in vivo* (Bernet *et al.*, 1994; Bernet-Camard *et al.*, 1997). The latter properties can be based either on the strain ability to adhere to the gastrointestinal tract (Bernet *et al.*, 1994), or on the secretion of (an) antimicrobial component(s) active *in vitro* and *in vivo* (Bernet-Camard *et al.*, 1997), or on both. Studies using the human intestinal cell line Caco-2 demonstrated the inhibitory effect of La1 organisms against the cell association of several pathogens found in human diarrhea, including enterotoxigenic *Escherichia coli* (ETEC), enteropathogenic *E.coli* (EPEC), and *Salmonella typhimurium* strains (Bernet *et al.*, 1994). All these three pathogenic species are known to interact with apical surfaces of HT-29 and/or Caco-2 intestinal cells, leading to morphological events nearly identical to those seen *in vivo* (Neeser *et al.*, 1989; Finlay and Falkow, 1990; Canil *et al.*, 1993).

Regarding now the molecular species involved in the adherence of lactic acid bacteria to the intestinal mucosa, the topic remains poorly documented. The importance of a glycolipid class, the lipoteichoic acids (Ofek *et al.*, 1975; Beachey and Ofek, 1976; Pinto *et al.*, 1983; Sherman and Savage, 1986; Teti *et al.*, 1987a,b) and/or cell surface proteins (Toba *et al.*, 1995) as mediators of adhesion of *Lactobacillus* spp. or other Gram-positive bacteria to human epithelial cells has been demonstrated. For *L.johnsonii* La1, the first hypothesis of an adhesion mechanism mediated by a bacterial "bridging" protein (Bernet *et al.*, 1994) has been revised, after having established the key role played by the cell surface-associated La1 lipotechoic acid for the La1 attachment to Caco-2 cells (Granato *et al.*, 1999). On the other hand, the nature of the receptors present on the surface of mammalian intestinal cells has been even less documented. The identification of certain glycolipids of the rat intestinal mucosa as putative receptors for a rat *Lactobacillus casei* strain (Yamamoto *et al.*, 1996), and the necessary presence of carbohydrate determinants on the surfaces of cultured human intestinal cells (HT-29) for the binding of two *Lactobacillus plantarum* strains (Adlerberth *et al.*, 1996) have suggested the occurrence of adhesion mechanisms involving complex carbohydrates. In this context, our first aim was here to identify the carbohydrate-binding

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specificities of *L.johnsonii* La1 by testing soluble oligosaccharides and glycoconjugates as adhesion inhibitors. As Caco-2 cells display typical features of an enterocytic differentiation (Pinto *et al.*, 1983), bacterial binding to these cells was studied in the presence of such potential inhibitors. It should be noted that the adhesion properties of *L.johnsonii* La1 onto Caco-2 intestinal cells have previously been shown to be dependent of the state of enterocytic differentiation of the cells (Bernet *et al.*, 1994). Thus, these cells were cultured accordingly, in order to assure the expression of the required receptor(s) for La1. In parallel, we examined the attachment of *L.johnsonii* La1 to glycolipids extracted from different sources and compared them to those already known to be specifically recognized by enteropathogens (Karlsson, 1989; Sporsem Oro *et al.*, 1990; Jagannatha *et al.*, 1991; Wenneras *et al.*, 1995). As a result, we identified here two major carbohydrate-binding specificities of *L.johnsonii* La1, a first one mannose-specific, and a second one revealed by a strong affinity for the gangliotetraosylceramide (GA1 = asialo-GM1) glycolipid. These findings put a new light on the way by which probiotic bacteria can provide protection to the gut against microbial pathogens, since both these specificities are equally shared by several enteropathogens, the latter using them for adhering to the host gut mucosa.

Results and discussion

Inhibition of the adhesion of L.johnsonnii *La1 to Caco-2 cell monolayers*

Recent studies on the nature of the receptor molecules responsible for the attachment of bacteria to mammalian epithelial cells indicate that host cells expose binding structures belonging to various molecular families. In some cases, binding determinants may be carried by (glyco)proteins, whilst in other cases, bacteria attach to glycolipids (Finley and Cossart, 1997). Consequently, we first tested the possible effect of different glycoproteins, glycolipids and oligosaccharides at the concentration of 3 mg/ml, on the adhesion of metabolically labeled La1 to Caco-2 cells. In the conditions used here, experiments in the absence of potential inhibitors gave adhesion scores around 10% (namely ~10^7 adhering bacterial cells per well plate). At confluence, well plates contained between 10^6 and 5×10^6 Caco-2 cells. Thus, it can be deduced that a typical adhesion experiment retained between 2 and 10 bacteria per intestinal cell, a number which excludes saturation. Moreover, it has been previously established that at pH 5, artifacts due to microaggregation are minimized (Granato *et al.*, 1999). A typical microscopical examination of the La1 bacteria bound to Caco-2 cells under such conditions is shown on Figure 1.

Mean values of adhesion inhibition scores are reported on Table I, for all experiments. First, the inhibition obtained with the mixture of newborn meconium glycolipids was significantly ($p < 0.05$) different and clearly more potent than inhibition scores obtained with all other probes (except the Endo-H-treated *mnn9* yeast mannoprotein). In addition, this effect was evidently dose-dependent (Table II). The potent inhibitory potential of this glycolipid mixture together with its extreme structural complexity prompted us to study further the La1 binding affinities to known glycolipids separated and immobilized on

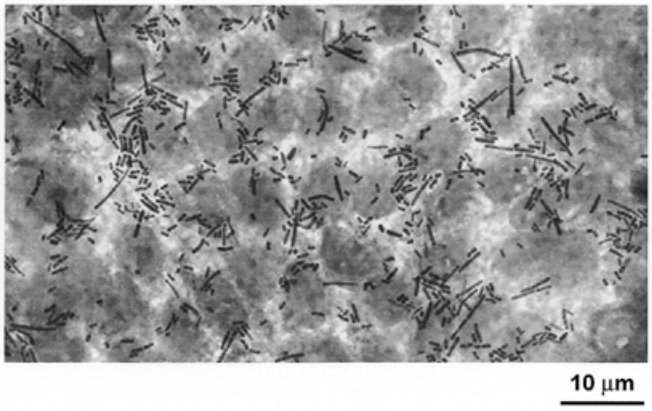

Fig. 1. Light microscopy of *Lactobacillus johnsonnii* (formerly *acidophilus*) La1 adhering onto Caco-2 intestinal cell monolayers.

Table I. Effect of various carbohydrates and glycoconjugates (3mg/ml) on the adhesion of *Lactobacillus johnsonnii* (formerly *acidophilus*) La1 to Caco-2 cell monolayers

	Adhesion Inhibition (%)	M[a]	MED[b]	Logit [c)] M′	M′ – 2SEM′	M′ + 2SEM′
New born human meconium glycolipids	48.6 / 74.8 / 50.4	57.9	50.4	**58.6**[d]	40.4	74.8
Endo-H treated mannoprotein from *S.cerevisiae mnn*9 mutant	34.3 / 69.1 / 67.7	57.0	67.7	**57.4**[d]	34.3	77.7
α-Methyl-mannoside	13.4 / 15.0 / 4.4	10.9	13.4	**9.7**	4.4	20.2
BSA	≤3[e] / 19.2	11.1	11.1	**7.9**	1.10	39.7
Fetuin	21.7 / ≤3	12.4	12.4	**8.5**	1.02	45.3
Lactose	21.0 / ≤3 / 10.0	11.3	10.0	**8.8**	2.71	25.3
N-Acetyllactosamine	4.5 / ≤3	3.8	3.8	**3.7**	2.44	5.5
Fucosyllactose	≤3 / 29.2 / ≤3	11.7	3.0	**6.8**	1.29	29.2
N-Acetylneuraminyl(α2–3)lactose	17.9 / 28	23.0	23.0	**22.6**	14.03	34.2

[a]M = mean (average).
[b]MED = median.
[c]Logit transformation: y = logit (p) = log (p / (1 - p)); p = antilog (y) / (1 + antilog(y))
[d]Both these M′ values are significantly different from all others (not contained in their 95% confidence interval ($p < 0.05$)).
[e]Values between 0 and 3 cannot be distinguished. The value of 3 was retained for the logit transformation.

TLC plates (Karlsson, 1989; see *Binding of* L.johnsonii *La 1 on thin-layer chromatograms*).

Binding studies done in the presence of a mannose glycoside, and of a glycoprotein bearing oligomannosides led to the following data (Table I): Methyl-α-D-mannoside, a specific monosaccharide glycoside able to inhibit *E.coli* adhesion mediated by type 1 pili (Firon *et al.*, 1983; Neeser *et al.*, 1986), was unable to significantly inhibit the adhesion of La1 to Caco-2 cells. Then, the yeast cell wall mannoprotein from the *mnn9* mutant of *Saccharomyces cerevisiae* was tested, after treatment with Endo-H. Indeed, structural studies indicated that *N*-linked oligomannosides from this *mnn9* mannoprotein expose mainly Manα1–3Man terminal sequences, whereas *O*-linked sugar chains expose mainly Manα1–2Man terminal sequences (Ballou, 1982). Since Manα1–3Man precisely corresponds to the "natural" high affinity ligand of *E.coli* type 1 pili (Neeser *et al.*, 1986), we were prompted to selectively remove the sugar chains containing such sequences. Thus, after treatment with Endo-H which cleaves *N*-linked chains only, this mannoprotein can be considered as a probe carrying *O*-linked oligomannosides, terminated mainly by the Manα1–2Man carbohydrate sequence. The convincing adhesion inhibition data obtained with this "modified" mannoprotein can be compared with the negative results yielded by methyl-α-D-mannoside (see Table I). However, an increase from 3 to 10 mg/ml of this glycoprotein concentration did not result in an increase of its inhibitory potency (Table II). This last finding pinpoints the presence of another adhesion mechanism which is not inhibited by the mannoprotein. Together, these data suggest for La1, a mannose-sensitive adhesion mechanism of a fine carbohydrate specificity different from that of *E.coli* type 1 pili. These findings may be compared with those of Adlerberth *et al.* (1996) who identified a mannose specific adhesin in *L.plantarum* 299 and 299v strains, and could show that these bacteria were able to agglutinate cells of *Saccharomyces cerevisiae* and bind to the human colonic cell line HT- 29 in an unknown mannose-sensitive manner. Similarly, our results showed an inhibition of adhesion in the presence of a *O*-glycosylated yeast mannoprotein, whose binding determinant was confirmed to be different from that of *E.coli* type 1 pili. Interestingly, Salmonella species are known to express different types of fimbriae, among which mannose-sensitive type 1 fimbriae seem to be the most common (Glegg and Swenson, 1994). Here also, it has long been known that the fine carbohydrate specificity of the Salmonella type 1 fimbriae is different from that of the *E.coli* type 1 pili (Firon *et al.*, 1983).

All other compounds tested (see Table I) were ineffective, which suggested a binding of La1 to Caco-2 cells inhibited either by specific glycolipids or by selected oligomannosides.

Binding of L.johnsonii *La1 on thin-layer chromatograms*

In a second step, we focused our attention on the binding of metabolically labeled *L.johnsonii* La1 to glycolipids extracted from different sources and separated on TLC plates according to the method of Karlsson (1987).

As seen in Table III, there was a consistent binding to gangliotriosyl- and gangliotetraosylceramide (also fucosylated gangliotetraosylceramide) and to lactotetraosylceramide (occurring in human meconium) and lactosylceramide. Some glycolipids ending in *N*-acetyllactosamine showed occasional binding and a number of glycolipids were consistently negative. Binding to asialo-GM1 (gangliotetraosylceramide) was very strong and has also been observed for a rat *L.casei* strain, another lactic acid bacteria (Yamamoto *et al.*, 1996), and for some species of Bifidobacteria by Fujiwara *et al.* (1997). When dealing with pathogenic strains, Sporsem Oro *et al.* (1990) showed by immuno-thin layer chromatography that asialo-GM1 was a binding structure for *E.coli* colonization factor antigens CFA/II and CFA/IV. Later, Wenneras *et al.* (1995) demonstrated by blotting experiments that the coli surface 3 (CS3) subcomponent of the colonization factor antigen II of enterotoxigenic *E.coli* was binding to electrophoretically separated and transblotted rabbit intestinal proteins, but that the binding could be inhibited by asialo-GM1 as well as by GM1 and GM2, but not GM3. This observation implied that the critical CS3 epitope consisted of the carbohydrate sequence GalNAcβ1–4Gal and that this sequence or a sequence having the same tertiary configuration was found in the blotted protein recognized by CS3. This sequence has also been implicated as a binding structure for enteropathogenic *E.coli* (Jagannatha *et al.*, 1991). In the case of La1, the highest binding to TLC plates was found with this sequence, but was not restrictive as can be seen with the lactosylceramides.

Table II. Dose/effect of two glycoconjugates mixtures on the adhesion of *Lactobacillus johnsonnii* (formerly *acidophilus*) La1 to Caco-2 cell monolayers

				Logit[c]		
	Adhesion inhibition (%)	M[a]	MED[b]	M′	M′ – 2SEM′	M′ + 2SEM′
Newborn human meconium glycolipids						
Dose:						
1 mg/ml	(9.5 / 4.6 / 33.8)	16	9.5	**12.1**	3.3	35.5
3 mg/ml	(48.6 / 74.8 / 50.4)	57.9	50.4	**58.6**	40.4	74.8
10 mg/ml	(78.8 / 71.2)	75.0	75.0	**75.2**	66.8	82.0
Yeast mannoprotein from mnn9 mutant, treated with Endo-H *(O*-glycosylated mannoprotein)						
Dose:						
1 mg/ml	(16.2 / 27.8)	22.0	22.0	**21.4**	12.0	35.2
3 mg/ml	(34.3 / 69.1 / 67.7)	57.0	67.7	**57.4**	34.3	77.7
10 mg/ml	(61.8 / 60.0 / 48.3)	56.7	60.0	**56.8**	48.2	64.9

[a, b, c] M, MED, and Logit transformation (as in Table I).

Table III. Binding of *Lactobacillus johnsonii* La1 to pure glycosphingolipids on thin-layer chromatograms

No.	Trivial name	Glycosphingolipid structure	Binding[a]	Source
A. Binding-active glycosphingolipids				
1.	LacCer d18:1-h16:0	Galβ4GlcβCer	+ (4/4)[b]	Dog small intestine
2.	Isotri	Galα3Galβ4GlcβCer	+ (4/4)[b]	Dog small intestine
3.	Gangliotri	GalNAcβ4Galβ4GlcβCer	+ (1/1)	Guinea pig erythrocytes
4.	Gangliotetra (= GA1 = Asialo-GM1)	Galβ3GalNAcβ4Galβ4GlcβCer	++ (5/5)	Mouse feces
5.	Fuc-gangliotetra	Fucα2Galβ3GalNAcβ4Galβ4GlcβCer	+ (1/1)	Mouse small intestine
6.	Neolactotetra (= LC4)	Galβ4GlcNAcβ3Galβ4GlcβCer	+ (1/4)	Human erythrocytes
7.	Lactotetra	Galβ3GlcNAcβ3Galβ4GlcβCer	+ (4/5)	Human meconium
8.		Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcβCer	+ (1/2)	Rabbit thymus
9.		NeuGcα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcβCer	+ (1/2)	Rabbit thymus
B. Non-binding glycosphingolipids				
1.	Sulfatide	SO_3-GalβCer	- (0/1)	Human meconium
2.	GM3	NeuGcα3Galβ4GlcβCer	- (0/1)	Horse erythrocytes
3.	Lactotri	GlcNAcβ3Galβ4GlcβCer	- (0/1)	
4.	Globotri (= CTH)	Galα4Galβ4GlcβCer	- (0/3)	Human erythrocytes
5.	Globotetra	GalNAcβ3Galα4Galβ4GlcβCer	- (0/2)	Human erythrocytes
6.	Forssman	GalNAcα3GalNAcβ3Galα4Galβ4GlcβCer	- (0/1)	Dog small intestine
7.	B-5	Galα3Galβ4GlcNAcβ3Galβ4GlcβCer	- (0/4)	Rabbit erythrocytes
8.	H5-2	Fucα2Galβ4GlcNAcβ3Galβ4GlcβCer	- (0/2)	Human erythrocytes
9.	B6-2	Galα3(Fucα2)Galβ4GlcNAcβ3Galβ4GlcβCer	- (0/2)	Human erythrocytes
10.	A6-2	GalNAcα3(Fucα2)Galβ4GlcNAcβ3Galβ4GlcβCer	- (0/1)	Human erythrocytes
11.	Le^a-5	Galβ3(Fucα4)GlcNAcβ3Galβ4GlcβCer	- (0/1)	Human small intestine
12.	Le^b-6	Fucα2Galβ3(Fucα4)GlcNAcβ3Galβ4GlcβCer	- (0/2)	Human small intestine
13.	SPG	NeuAcα3Galβ4GlcNAcβ3Galβ4GlcβCer	- (0/1)	Human erythrocytes
14.	Sulf-gangliotetra	SO_3-Galβ3GalNAcβ4Galβ4GlcβCer	- (0/2)	Mouse small intestine
15.		Galβ4GlcNAcβ6(Galβ4GlcNAcβ3)Galβ4GlcβCer	- (0/2)	Bovine buttermilk
16.		Galβ4GlcNAcβ6(NeuAcα6Galβ4GlcNAcβ3)Galβ4GlcβCer	- (0/2)	Bovine buttermilk

[a]++ marks a significant darkening on the autoradiogram when 4 µg of the glycosphingolipid was applied on the thin-layer plate, + marks a weak darkening, while – marks no darkening. The numbers in parenthesis give the number of positive binding/times tested.[b]Glycosphingolipid mixture utilized for testing.

In the present work, we have been able to identify two major carbohydrate-binding specificities of *L.johnsonii* La1. The first one (mannose-specific) is slightly different from that of *E.coli* type 1 pili (Firon *et al.*, 1983; Neeser *et al.*, 1986), as are those exhibited by Salmonella type 1 fimbriae (Firon *et al.*, 1983) and *L.plantarum* 299 and 299v adhesin (Adlerberth *et al.*, 1996). The second one mediates a strong affinity for the asialo-GM1 glycolipid, which has already been demonstrated for ETEC and EPEC strains (Sporsem Oro *et al.*, 1990; Jagannatha *et al.*, 1991; Wenneras *et al.*, 1995), as well as for a rat *L.casei* strain (Yamamoto *et al.*, 1996) and several bifidobacterial strains (Fujiwara *et al.*, 1997). To date, the presence of asialo-GM1 in human intestine has never been established but the presence of microorganisms from the normal flora able to desialylate GM1 may be assumed. However, the inhibitory activity of the human meconium glycolipid mixture observed earlier in this study is unlikely due to asialo-GM1, since newborn meconium is not exposed to a bacterial microflora during fetal life. Hence, the positive binding of La1 to other structures occurring in human meconium such as lactotetraosylceramide (see Table III), even if less strong, is probably a better explanation for the activity of such a glycolipid mixture. On the other hand, asialo-GM1 is abundantly found in the mouse intestine where it should act as a receptor for La1, enabling an already observed important colonization of the mouse gut (F.Rochat, unpublished observations) which in turn may protect the animal against infectious challenge (Bernet-Camard *et al.*, 1997). Thus, we have shown that *L.johnsonii* La1 can bind cell membrane molecules through mechanisms which are shared by other pathogenic and nonpathogenic bacteria. This observation could explain, at least partly, the La1 competitivity for pathogen adhesion onto Caco-2 cells (Bernet *et al.*, 1994). The asialo-GM1 glycolipid has also been implicated as a binding structure for other bacteria with tropism for non-intestinal tissues such as pulmonary tissue (Krivan *et al.*, 1988). This is true in particular for *Pseudomonas aeruginosa* whose binding to

asialo-GM1 is a tip-associated event mediated by the *C*-terminal region of its structural pilin subunit (Lee *et al.*, 1994). Thus, asialo-GM1 seems to be important for the colonizing capacity of many pathogenic and nonpathogenic bacteria at different human and animal mucosal surfaces. Finally, asialo-GM1 has been recognized as a specific marker of NK cells (Yu *et al.*, 1992). The interaction of La1 with this cell population may also be one of the molecular basis of the immunomodulatory activities of this probiotic bacterial strain (Haller *et al.*, 2000). Further studies are now needed to identify the receptor or receptors for La1 on the Caco-2 epithelial cells, and to assess the precise involvement of the La1-LTA in the cell–cell recognition mechanism or mechanisms.

Materials and methods

Inhibition of the adhesion of L.johnsonnii *La1 to Caco-2 cell monolayers*

Adhesion of *L.johnsonii* La1 onto Caco-2 cells was studied as follows: *L.johnsonii* La1 bacteria were grown in anaerobic conditions in Man, Rogosa, Sharpe (MRS) broth (Difco) overnight at 37°C in the presence of 10 μCi of tritiated adenine/ml (21 Ci/mmol, Amersham). Caco-2 cells (American Type Culture Collection, USA) were routinely grown in Dulbecco modified Eagle's minimal essential medium (Gibco, BRL) supplemented with 20% heat-inactivated fetal serum (Boehringer) and 1% nonessential amino acids (Gibco, BRL) as described previously (Bernet *et al.*, 1994). Cells were used at post-confluence after 20 days of culture (fully differentiated state; Pinto *et al.*, 1983).

For adhesion assays, metabolically labeled bacteria were washed three times with PBS and resuspended at a concentration of 10^8 cfu/ml in 0.05 M acetate buffer pH 5 containing 0.15 M NaCl (a solution 0.05 M sodium acetate and 0.15 M sodium chloride was adjusted to pH 5.0 with glacial acetic acid); 1 ml of the bacterial suspension was incubated at 37°C for 1 h onto Caco-2 cells (6 wells plates) which had been previously washed with acetate buffer. Afterwards, supernatants were removed and cells were washed three times with the same buffer. Cell monolayers were disrupted by addition of 1 ml of 1 M NaOH. The lysate and a 0.5-ml wash were transferred in a counting vial containing 1 ml of benzethonium hydroxide (Sigma). Vials were put in an incubator at 60°C for 1 h, cooled, and counted after the addition of 10 ml of Ultima Gold (Packard). Each experiment was performed in triplicate and mean values were calculated.

For adhesion inhibition assays, the compounds to be tested were dissolved in acetate buffer, then mixed with the bacterial suspension and incubated on Caco-2 cells as described above. Experiments were repeated twice or three times (always involving triplicates) for each test compound. The logit transformation was chosen as the best tool for statistical calculations (better than the use of a mean or a median), since it takes into account the limitation of the inhibition rate between 0 and 100% only.

Human newborn meconium glycolipids and glycoproteins were separated as described by Hounsell *et al.* (1985). The preparation of the samples to be used for testing concentrations of 1 mg/ml and 3 mg/ml of this glycolipid mixture in acetate buffer yielded clear solutions, whereas the most concentrated suspensions (10 mg/ml) required a centrifugation step before mixing with the bacteria. *Saccharomyces cerevisiae mnn 9* mutant was a kind gift of C.E.Ballou (Department of Biochemistry, University of California, Berkeley). The isolation of the cell wall mannoprotein was done according to Frevert and Ballou (1985). Briefly, anion-exchange chromatography on DEAE-Sephadex A-50 using a 0.3 M sodium chloride solution as an eluent yielded a large retained fraction which was collected and further homogenized to purity on a hydroxyapatite gel. The release of the *N*-linked sugar chains was performed by a treatment with endo-β-*N*-acetylglucosaminidase-H (Endo-H), as described by Tsai *et al.* (1986). This enzymatic treatment was repeated twice and the removal of *N*-glycans was followed by high-performance thin layer chromatography: under such conditions, no more *N*-glycan chains were released at the end of the second incubation period. The resulting mannoprotein was finally purified by liquid chromatography on a Sephadex G-50 column (Pharmacia, Sweden). The other tested compounds were from Sigma Chemical Co. (St. Louis, MO).

Binding of L.johnsonii *La1 on thin-layer chromatograms*

Briefly, total acid and nonacid glycolipid fractions, from the sources given in Table III, were isolated as described (Karlsson, 1987). The pure glycolipids used in the binding studies were isolated by repeated chromatography of native glycolipids, or acetylated derivatives, on silicic acid columns (Iatrobeads 6RS-8060, Iatron Laboratories Inc., Tokyo, Japan) or by HPLC on silicic acid columns. The isolated glycolipids were characterized by mass spectrometry (Samuelsson *et al.*, 1990), proton NMR spectroscopy (Falk *et al.*, 1979a–c; Koerner *et al.*, 1983), and degradation studies (Yang and Hakomori, 1971; Stellner *et al.*, 1973).

Binding of ^{35}S-labeled *L.johnsonii* La1 to glycolipids on thin-layer chromatograms was examined as follows: *L.johnsonii* bacteria were cultured in MRA broth supplemented with 50 μCi ^{35}S-methionine/10 ml (Amersham, UK) at 37°C overnight under anaerobic conditions. The bacteria were collected by centrifugation, and washed three times with phosphate-buffered saline (PBS), pH 7.3. The bacteria were thereafter suspended in PBS to approximately 1×10^8 CFU/ml. The specific activities of the suspensions were approximately 1 c.p.m. per 100 bacterial cells. The chromatogram binding assay was done as described previously (Hansson *et al.*, 1985). Glycolipid mixtures (20–40 μg/lane), or pure glycolipids (1–4 μg/lane), were separated on glass- or aluminum-backed silica gel 60 HPTLC plates (Merck, Darmstadt, Germany), using chloroform/methanol/water (60:35:8, by volume) as solvent system. The dried chromatograms were dipped in 0.3–0.5% (w/v) polyisobutylmethacrylate (Plexigum P28, Röhm, GmbH, Darmstadt, Germany) in diethylether/*n*-hexane (1:5, by volume) for 1 min, and air-dried. Blocking of unspecific binding sites was done by immersing the plates in PBS containing 2% bovine serum albumin (w/v) and 0.1% Tween 20 (w/v) for 2 h at room temperature. Thereafter, suspensions of radiolabeled bacteria (diluted in PBS to 1×10^8 CFU/ml and $1–5 \times 10^6$ c.p.m./ml) were gently sprinkled over the chromatograms, and incubated for 2 h at room temperature. After washing six times with PBS, and drying, the thin-layer plates were autoradiographed for 12–48 h using XAR-5 x-ray films (Eastman Kodak, Rochester, NY).

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