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Safe as mother's milk: Carbohydrates as future anti-adhesion drugs for bacterial diseases

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The majority of infectious diseases are initiated by adhesion of pathogenic organisms to the tissues of the host. In many cases, this adhesion is mediated by lectins present on the surface of the infectious organism that bind to complementary carbohydrates on the surface of the host tissues. Lectin-deficient mutants often lack ability to initiate infection. Soluble carbohydrates recognized by the bacterial lectins block the adhesion of the bacteria to animal cells *in vitro*. Moreover, they have also been shown to protect against experimental infection by lectin-carrying bacteria in different organs of mammals such as mice, rabbits, calves and monkeys.

In a phase II clinical trial, a pentasaccharide shown to have anti-adhesive activity against *Streptococcus pneumoniae* and *Hemophilus influenzae in vitro* failed to protect young children from nasopharyngeal colonization with these organisms and from developing otitis media. This could be because insufficient drug was delivered via nasal spray, because bacteria express multiple specificities, the inhibition of which may require a cocktail of oligosaccharides, or because children have different carbohydrate receptors from those of adults. The results of a clinical trial in which *N*-acetylneuraminyl(α2-3)lactose was administered orally to *Helicobacter pylori* positive patients in an effort to reduce or eradicate bacterial colonization, are awaited with interest.

Although the high cost of production of the required oligosaccharides is falling with the recent introduction of enzymatic methods of synthesis, new technologies, in particular the use of engineered bacteria, promise to lower it even further. Attachment of the oligosaccharides to soluble polymeric carriers will increase greatly their effectiveness as anti-adhesion agents. There is no doubt that anti-adhesive oligosaccharides will in the near future join the arsenal of drugs for the therapy of bacterial diseases.

Keywords: **fimbriae, glycolipids, glycoproteins, infection, lectins, oligosaccharides**

Introduction

The alarming increase in antibiotic resistant bacterial pathogens makes it imperative to intensify the search for new means of combating such bacteria. A highly promising approach is anti-adhesion therapy, namely the use of agents, in particular carbohydrates, that prevent the attachment or adhesion of the bacteria to host tissues, or detach them from the tissues at the early stages of infection. From a medical point of view, such molecular intervention may be considered mild and gentle, and more sound ecologically, as well as safer, compared with present chemotherapy approaches. Saccharides are ideal for this purpose as they are unlikely to be toxic or immunogenic, in particular since many of those that inhibit bacterial adhesion are normal constituents of cell surfaces or body fluids, especially of human milk. Moreover, because saccharides are not bactericidal, selection of resistant strains is unlikely to occur, thus reducing considerably the spread of such strains in the environment.

The scientific basis for the hope that carbohydrates would prove to be, in the not too distant future, effective anti-adhesion drugs against bacterial diseases, is founded on a combination of widely documented findings (for reviews, see [1–6]): (1) adhesion of bacteria to host tissues is a prerequisite for most infections to occur; (2) frequently it is mediated by bacterial surface lectins and is inhibited by the mono- and oligosaccharides for which the lectins are specific, as well as by antibodies to the lectins or their receptors; (3) such saccharides, and the anti-lectin antibodies, protect animals against experimental infection by the lectin-carrying bacteria.

In the following, we review in some detail the findings supporting the above conclusions.

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Adhesion is prerequisite for infection

The majority of infectious diseases are initiated by adhesion of pathogenic organisms to cells and mucosal surfaces of the host [1]. Adhesion is required so that the organisms avoid being swept away by the natural cleansing mechanisms of the host, such as airflow in the respiratory tract or urine flow in the urinary tract (Figure 1). It also provides the pathogens with better access to sources of nutrition, facilitates the delivery of toxic agents into the host tissues and eventually the penetration of the bacteria into the tissues.

Adhesion is mediated by bacterial surface lectins and is inhibited by sugars

The adhesion of many pathogenic organisms is mediated by lectins present on the surface of the organisms, that bind to complementary carbohydrate constituents of glycoproteins or glycolipids on the surface of the host tissues (Table 1). By virtue of their ability to mediate adhesion to host tissues, the lectins can be considered as bacterial virulence factors. Thus, lectin-deficient mutants of the pathogens are often unable to initiate infection. For example, analysis of the development of urinary tract infection in monkeys challenged with lectin-positive versus lectin-negative *E. coli* P strains (Galα4Gal-specific) has shown conclusively that the presence of a single lectin is necessary and sufficient to direct the pathogen to the kidney and to induce disease [7]. Similarly, it has been established that the type 1, mannose-specific lectin of other strains of *E. coli* increases the virulence of the bacteria in the urinary tract by promoting bacterial persistence and by enhancing the inflammatory response to infection [8].

The bacterial surface lectins are frequently in the form of fimbriae (or pili), elongated and relatively rigid submicroscopic structures. They are generally polymorphic, and consist of an assembly of hundreds of protein subunits of several kinds, only one kind of which binds carbohydrates [1]. Fimbriae are well suited to serve as mediators of adhesion of the bacteria to the target tissues. For instance, those of the uropathogenic *E. coli* are mechanically resilient to the cleansing action of urine flow that removes other bacteria.

Until the early 1980's, only bacteria specific for mannose were known, namely the type 1 fimbriated strains of urinary tract infections. Since then, *E. coli* strains with many different specificities were discovered (Table 1). They include urinary strains carrying P fimbriae that are specific for galabiose [Gal(α1-4)Gal], and neural S fimbriated strains specific for NeuAc(α2-3)Gal(β1-3)GalNAc. Bacteria specific for other sugars have been identified, e.g. *Neisseria gonorrhoea*, a genital pathogen, that recognizes lactose. Recently, much attention is focused on *Helicobacter pylori*, the causative agent of peptic ulcer, that expresses more than ten separate binding specificities [6]. Several of these lectins recognize NeuAc(α2-3)Gal(β1-4)Glc (Sia3′Lac) and its *N*-acetylglucosamine analog (Sia3′LacNAc) while others are specific for the Le^b determinant Fuc(α1-2)Gal(β1-3)[Fuc(α1-4)]GlcNAc, both common constituents of human milk. An individual bacterium may co-express more than one lectin, e.g. certain strains of *E. coli* are both mannose and galabiose specific and those of *H. pylori* recognize simultaneously the three milk constituents just mentioned. Another complicating factor is that lectin expression is dependent on growth conditions [9].

Bacteria recognize not only terminal nonreducing sugars but also internal ones, as demonstrated originally with P

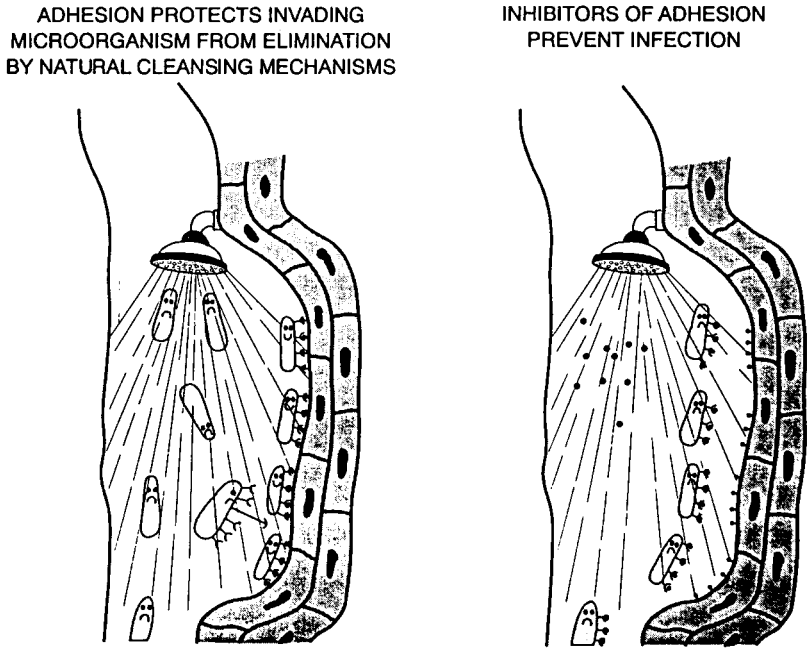

Figure 1. Microbial adhesion and anti-adhesion therapy (adapted from the PhD, thesis of Dina Zafriri, Tel Aviv University, 1988).

Table 1. Carbohydrates as attachment sites for bacterial pathogens on animal tissues.

Organism	*Target tissue*	*Carbohydrate*	*Form**
E. coli Type 1	Urinary	Man(α1-3)[Man (α1-6)] Man	GP
P	Urinary	Gal(α1-4)Gal	GSL
S	Neural	NeuAc(α2-3)Gal(β1-3)GalNAc	GSL
CFA/1	Intestinal	NeuAc(α2-8)-	GP
K1	Endothelial	GlcNAc(β1-4)GlcNAc	GP
K99	Intestinal	NeuGc(α2-3)Gal(β1-4)Glc**	GSL
H. influenza	Respiratory	$[NeuAc(\alpha2\text{-}3)]_{0,1}$Gal(β1-4)GlcNAc-(β1-3)Gal(β1-4)GlcNAc**	GSL
H. pylori	Stomach	NeuAc(α2-3)Gal(β1-4)Glc(NAc)**	GP
		Fuc(α1-2)Gal(β1-3)[Fuc(α1-4)] Gal**	GP
K. pneumoniae	Respiratory	Man	GP
M. pneumoniae	Respiratory	NeuAc(α2-3)Gal(β1-4)Glc(Nac)**	GP
N. gonorrhoea	Genital	Gal(β1-4)Glc(NAc)**	GSL
N. meningitidis	Respiratory	$[NeuAc(\alpha2\text{-}3)]_{0,1}$Gal(β1-4)GlcNAc-(β1-3)Gal(β1-4)GlcNAc**	GSL
P. aeruginosa	Respiratory	Gal(β1-3)Glc(NAc)(β1-3)Gal(β1-4)-Glc**	GSL
S. typhimurium	Intestinal	Man	GP
S. pneumoniae	Respiratory	$[NeuAc(\alpha2\text{-}3)]_{0,1}$Gal(β1-4)GlcNAc-(β1-3)Galβ(1-4)GlcNAc**	GSL
S. suis	Respiratory	Gal(α1-4)Gal(β1-4)Glc	GSL

*Predominant form in tissue: GP, glycoproteins; GSL, glycolipids.
**Structures present in human milk oligosaccharides.

fimbriated *E. coli* that bind to galabiose even when its nonreducing galactose moiety is substituted at the 3-OH by e.g. GalNAcβ [9]. A recent example is *Streptococcus pneumoniae*, that interacts specifically not only with thc pentasaccharide NeuAc(α2-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc, but also with the corresponding internal tetrasaccharide Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc and trisaccharide GlcNAc(β1-3)Gal(β1-4)Glc [10,11].

E. coli K99 provides a striking illustration of the fine carbohydrate specificity of bacterial surface lectins, and its relationship to the animal tropism of the bacteria [6]. This organism binds to glycolipids containing *N*-glycolylneuraminic acid (NeuGc), in the form of NeuGc(α2-3)Gal(β1-4)Glc, but not to those that contain *N*-acetylneuraminic acid. These two sugars differ in only a single hydroxyl group, present in the acyl substituent on the 4-NH group of *N*-glycolylneuraminic acid and absent in that of *N*-acetylneuraminic acid. *N*-Glycolylneuraminic acid is found on intestinal cells of newborn piglets, but it disappears when the animals develop and grow. It is also not formed normally by humans. This explains why *E. coli* K99 can cause diarrhea (often lethal) in piglets, but not in adult pigs nor in humans.

Soluble carbohydrates recognized by the bacterial lectins block adhesion of the bacteria to a variety of animal cells *in vitro*. This is illustrated in Figure 2 that shows the inhibition by Sia3′Lac of *H. pylori* binding to duodenum-derived human cells. Such saccharides can also detach bacteria that are already bound to cells via their surface lectins (Figure 3). Since several of the inhibitory oligosaccharides are common constituents of human milk, it was concluded that these substances play a protective role against different infectious agents in breastfed babies [13,14]. This is the basis of attempts

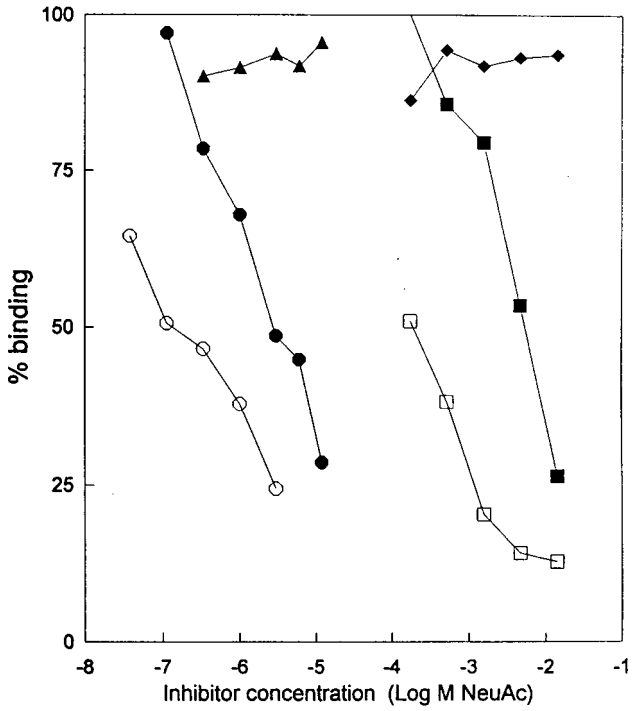

Figure 2. Inhibition by mono- or multivalent Sia3′Lac of the adhesion of *H. pylori* to monolayers of gastrointestinal epithelial cells (Hep-2, closed symbols; HuTu, open symbols). Bacteria were preincubated with free Sia3′Lac (squares), with HSA (human serum albumin) derivatized with 20 mole per mole of the trisaccharide (circles), with unconjugated HSA (triangles) or with Sia6′Lac (diamonds). The concentration of underivatized HSA is plotted to correspond to its molar concentration of HSA in the Sia3′Lac-HSA conjugate. (From Ref. [12], reproduced with permission).

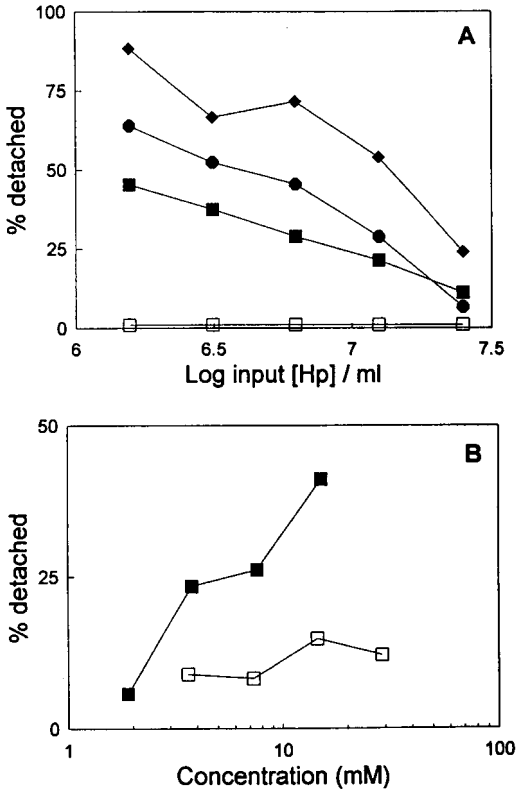

Figure 3. Detachment of bound bacteria by Sia3′Lac. *H. pylori* CP22 (3×10^7/ml) were incubated with HuTu-80 monolayers for 15 min, and unbound bacteria were removed by three washes of buffer alone or buffer containing the indicated compounds: (a) Sia3′Lac, 2 mg/ml (3 mM, closed squares); lactose 2 mg/ml (5.8 mM, open squares); gastric mucin, 2 mg/ml per ml (closed circles); a mixture of 3′SL and gastric mucin (2 mg/ml each, diamonds). The greatest detachment was obtained at lower bacterial loads. (B) The detaching effect of Sia3′Lac (closed squares) is concentration dependent, while lactose (open squares) has no effect (bacterial input, 3×10^7/ml). (From Ref. [12], reproduced with permission).

made by one biotechnology company to develop oligosaccharides for use in nutritional and other non-prescription products [15].

The affinity of sugars for lectins is usually low, in the millimolar range. An increase of several orders of magnitude in the affinity can be achieved by suitable chemical derivatization. Thus, hydrophobic α-mannosides, e.g. of methylumbelliferyl or *p*-nitrochlorophenyl, were 500–1000 times more inhibitory than methyl α-mannoside of the adhesion of type 1 fimbriated *E. coli* to yeasts or to rabbit ileal epithelial cells [16].

Marked increase in affinity of the inhibitors for the bacterial lectins is also obtained by their attachment to polymeric carriers, to form multivalent ligands, as demonstrated with *H. pylori* (Figure 2).

Inhibition of adhesion can also he achieved by suitable antibodies, e.g. by anti-type 1 fimbriae or anti-P fimbriae antibodies, as well as by antibodies directed against the sugars for which the lectins are specific [1]. A recent example is that of antibodies against FimH, the mannose binding subunit of the type 1 fimbrial lectin, which prevented the adhesion to a human bladder cell line of *E. coli* that expresses the lectin [17].

Anti-adhesive sugars, in addition to blocking the attachment of the bacteria to tissues, may act similarly on the binding of toxins such as those of *Shigella dysenteriae* type 1, or of the homologous toxins of *E. coli*, also called Verotoxins, that are Gal(α1-4)Gal-specific. In this system too, polyvalent ligands, when appropriately designed, are markedly more inhibitory than monovalent ones; while the latter are active at the millimolar level only, the former are active at the nanomolar level [18].

Carbohydrates act as anti-adhesion drugs in experimentally induced infections

The ability of sugars to protect animals against experimental infection by lectin-carrying bacteria has been first reported by our group two decades ago, in a model of urinary tract infection in mice [19]. We found that coadministration of methyl α-mannoside with type 1 fimbriated *E. coli* into the urinary bladder of the mice reduced the rate of urinary tract infection by two thirds, while methyl α-glucoside, which is not inhibitory to the fimbriae, was without effect. The protective effect of anti-adhesive sugars was subsequently demonstrated in a variety of studies with different pathogenic bacteria and animals (Table 2). For instance, colostrum-deprived newborn calves, which had been given a lethal dose of *E. coli* K99 could be cured by drinking water containing glycopeptides prepared from cow plasma non-immunoglobulin glycoproteins [20]. The treatment decreased by 100 fold the number of bacteria that adhered to the intestinal epithelial cells of the calves. Although the glycopeptides used were not analysed in

Table 2. Inhibitors of carbohydrate-specific adhesion prevent bacterial infection *in vivo**.

Bacterial pathogen	*Animal and site*	*Inhibitor*
E. coli type 1	Mouse UT	MeαMan
	Mouse GIT	Mannose
	Mouse UT	Anti-Man-antibody
E. coli P	Mouse UT	Globotetraose
	Monkey UT	Gal(α1-4)GalβOMe
E. coli K99	Calf GIT	Glycopeptides
H. pylori	Piglet GIT	Sia3′LacNAc
	Monkey GIT	Sia3′Lac
K. pneumoniae type 1	Rat UT	MeαMan
Shigella flexneri type 1	Guinea pig eye	Mannose
S. pneumoniae	Mouse lung	*N*-Acetyglucosamine
	Rabbit lungs	Lacto-N-neotetraose

UT, Urinary tract; GIT, gastrointestinal tract.
*For literature not discussed in text, see Sharon, N. in ref. [4].

detail, it is very likely that they contained the NeuGc(α2-3)Gal-R sequence recognized by the bacteria.

In rabbits and infant rats, experimental pneumonia caused by *S. pneumoniae* was markedly reduced by intranasal (Figure 4) or intratracheal administration of either free oligosaccharides or as neoglycoproteins [11].

Very recently, results of the treatment of twelve *H. pylori*-positive rhesus monkeys with the sodium salt of Sia3′Lac alone or in combination with either one of the commonly used anti-ulcer drugs, bismuth subsalicylate (a gastric coating agent) and omeprazole (a proton pump inhibitor) have been reported [21]. Of the six monkeys that were given the trisaccharide only, two were cured permanently, and a third animal was transiently cleared, while three of the animals remained persistently colonized. The other six animals received the trisaccharide with the anti-ulcer drugs in different combinations. Transient decreases in colony counts were observed in three of these animals, but gastritis was suppressed only in the two animals that became persistently *H. pylori* negative. No side effects were observed in any of the animals receiving Sia3′Lac. The authors concluded that 'anti-adhesive therapy is safe; it can cure or decrease *H. pylori* colonization in some rhesus monkeys, but the addition of a proton pump inhibitor or bismuth subsalicylate does not increase cure rate'.

Clinical experience

Clinical experience in humans with oligosaccharides as anti-adhesive drugs is still very limited. A phase II clinical trial in which over 500 children 10–24 months old were treated prophylactically with the anti-adhesive pentasaccharide, Sia3′LNnT, administered by nasal spray (2 mg in each nostril twice per day) for 3 months failed to reduce the incidence of nasopharyngeal colonization with *Streptococcus pneumoniae* and *Hemophilus influenzae* and of acute otitis media [22]. This failure could be due to the fact that during natural infection, bacteria express multiple lectins with diverse specificities, the inhibition of which may require a cocktail of oligosaccharides. Alternatively, the amount of drug and/or frequency of administration may not have been sufficient to achieve a therapeutic effect. Because carbohydrate expression on cell surfaces (e.g. Lewis and Ii blood group determinants on red blood cells) may change during early development, the possibility exists that the display of adhesion targets on upper respiratory epithelium of young children differs from that of adults from whom upper respiratory target cells were obtained for *in vitro* preclinical anti-adhesion studies. The results of a clinical trial in which orally administered Sia3′Lac is being tested for its ability to clear gastric colonization by *H. pylori*, are awaited with interest.

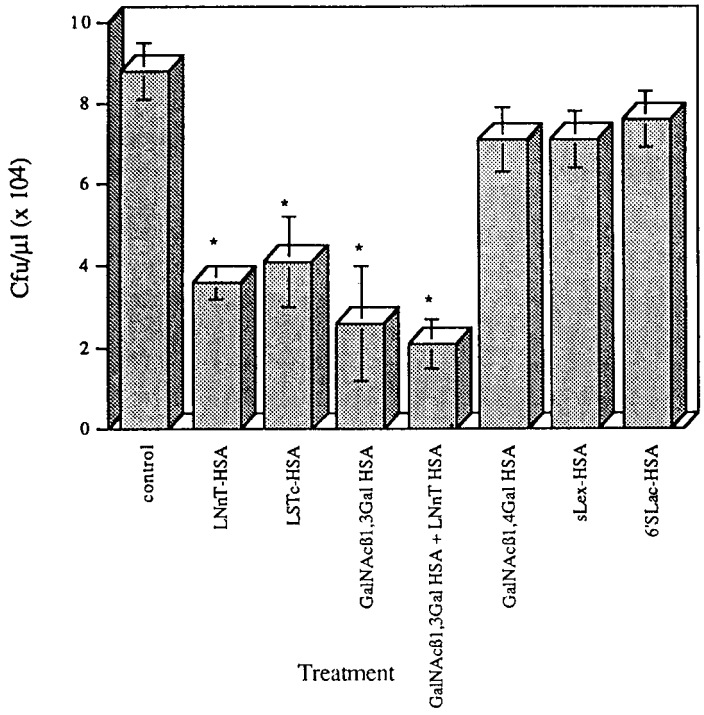

Figure 4. Effect of oligosaccharides conjugated to human serum albumin (HSA) on colonization of nasopharynx of infant rats by pneumococci. *S. pneomoniae* (type 3) were incubated without or with the conjugates (100 mM carbohydrate, 15 or 20 mole per mole protein) the in saline at room temperature for 15 min and then inoculated intranasally into the rats (10^6 bacteria per animal). The number of viable bacteria recovered in nasal washes was determined after 3 h. Values are mean ± SD for groups of 4 animals; experiment was performed twice. *Significantly different from control ($p < 0.1$). (From Ref. [11], reprinted with permission.)

Concluding remarks

The presence of multiple lectins with distinct sugar specificities that are encoded by DNA often located on the same chromosome of the pathogen, is most likely the major impediment for the use of sugars as anti-adhesion drugs. To overcome this problem, it is necessary to learn more about the lectins of the bacterial pathogens and the factors affecting their expression in the course of natural infection. This will allow the preparation of suitable cocktails of inhibitory sugars for the treatment of bacterial infections, instead of the single sugars in use until now. The low affinity of free saccharides for the bacterial lectins is another stumbling block, which may be overcome by their attachment to polymeric carriers or presentation as dendrimers [23]. Production of the oligosaccharides is still extremely costly, and to act effectively, large doses are required. However, new technologies, in particular the use of engineered bacteria [24], promise to lower this cost markedly. Another possibility is to develop suitable carbohydrate analogs (glycomimetics) that are more potent inhibitors of bacterial adhesion agents than the presently available saccharides.

In conclusion, there is little doubt that in spite of these and other problems, there are compelling reasons to believe that this novel means of therapy of microbial diseases has a bright future, and that it will soon move from the realm of dreams to reality.

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