

Bioengineered bugs expressing oligosaccharide receptor mimics

Toxin-binding probiotics for treatment and prevention of enteric infections

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Abbreviations: ETEC, enterotoxigenic *Escherichia coli*; Gal, galactose; GalNAc, *N*-acetyl galactosamine; Glc, glucose; GlcNAc, *N*-acetyl glucosamine; LPS, lipopolysaccharide; STEC, Shiga toxinogenic *E. coli*

Many microbial pathogens recognize oligosaccharides displayed on the surface of host cells as receptors for toxins and adhesins. These ligand-receptor interactions are critical for disease pathogenesis, making them promising targets for novel anti-infectives. One strategy with particular utility against enteric infections involves expression of molecular mimics of host oligosaccharides on the surface of harmless bacteria capable of surviving in the gut. This can be achieved in Gram-negative bacteria by manipulating the outer core region of the lipopolysaccharide (LPS) through expression of cloned heterologous glycosyltransferases. The resultant chimeric LPS molecules are incorporated into the outer membrane by the normal assembly route and presented as a closely packed 2-D array of receptor mimics. Several such "designer probiotics" have been constructed, and these bind bacterial toxins in the gut lumen with very high avidity, blocking their uptake by host cells and thereby preventing disease.

Introduction

Enteric infectious diseases continue to cause massive morbidity and mortality in humans, leading to over 2 million deaths each year. Effective vaccines are still not available for a number of important diarrheal diseases, and controlling these with conventional antimicrobial therapy is being complicated by increasing rates of drug resistance. A number of important enteric pathogens, such as *Vibrio cholerae*, Shiga toxinogenic *Escherichia coli* (STEC), enterotoxigenic *E. coli* (ETEC), *Clostridium difficile*, etc. secrete toxins or express adhesins that bind to host cells via oligosaccharide receptors displayed on surface glycolipids or glycoproteins. These pathogen-receptor interactions are

highly specific and are critical for pathogenesis. Moreover, the repertoire of glycans displayed by the host, as well as their distribution within various tissues, has a major influence on susceptibility to a given pathogen, its site of colonization, tissue tropism of toxins, and clinical manifestations of the resultant disease.¹ Accordingly, interference with the binding of a toxin or adhesin to its cognate host receptor is a promising anti-infective strategy. Blockade of adherence would prevent establishment of an infection altogether, while toxin neutralization would prevent development of symptoms until the organism is eliminated by local immune responses. Specific receptor structures recognized by key toxins and adhesins of a number of important human and animal enteric pathogens have already been characterised and this has facilitated development of receptor-blocking anti-infectives.² Of course, microbes are unlikely to develop resistance to such agents without compromising their capacity to recognize the target receptor and cause disease.

A number of studies have employed synthetic oligosaccharides corresponding to a given receptor epitope to competitively inhibit ligand binding.³ However, such interactions are often complicated, and may require either presentation of the oligosaccharide in a specific conformation, or multivalent interactions. Moreover, for enteric applications, digestive enzymes present in the small intestine may cleave free oligosaccharides, reducing their utility in more distal regions of the gut. Several enteric pathogens produce so-called AB₅ toxins, which bind to glycolipid receptors on host cells via a pentameric B subunit, each monomer of which engages at least one cognate host glycan.⁴ This leads to a cooperative, multivalent, high-avidity docking between the toxin and the host cell surface, which triggers holotoxin internalization. As a general rule, the free oligosaccharide components of the glycan receptors for these toxins are much less effective at blocking toxin-receptor interactions than either the glycan itself, or multivalent oligosaccharide conjugates.⁴⁻⁷ Effective synthetic toxin-binding agents usually comprise multiple oligosaccharide epitopes displayed on complex three dimensional scaffolds, often designed with reference to the crystal structures of the respective toxin binding pentamers in complex with their cognate glycans.⁸⁻¹⁰

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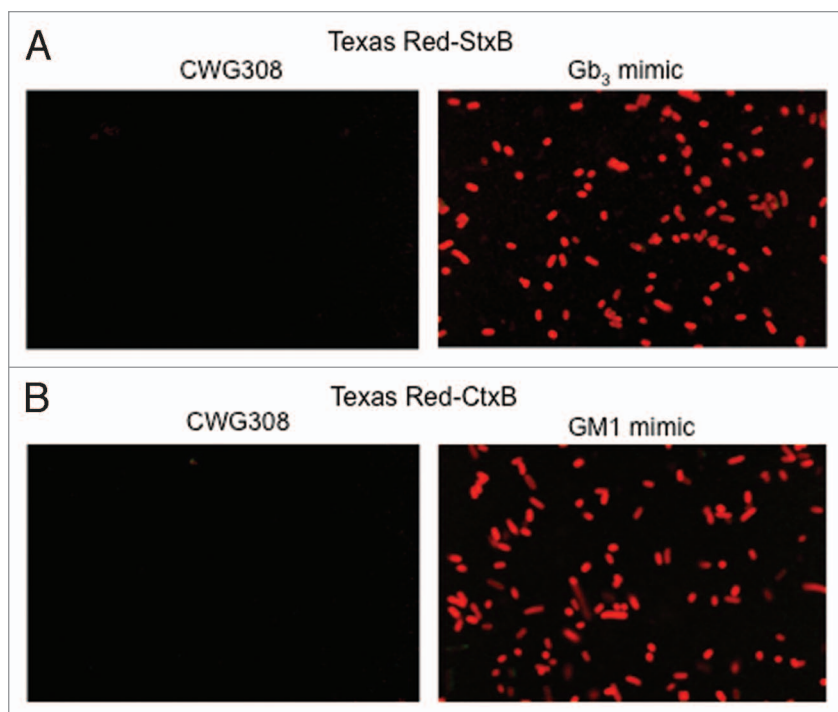


Figure 1. Specific binding of toxins to cognate receptor mimic probiotics. Suspensions of the *E. coli* host strain CWG308, or derivatives expressing mimics of Gb₃ (A) or GM1 (B), fixed on poly-L-lysine-treated slides, were reacted with Texas Red-labelled Shiga toxin B subunit (StxB) (A) or cholera toxin B subunit (CtxB) (B). Slides were examined using a BioRad MRC 600 Dual-Fluorescence Confocal microscope.

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Construction of Receptor Mimic Probiotics

We have developed an alternative, probiotic approach to blockade of enteric pathogen-receptor interactions, by engineering expression of molecular mimics of host receptor glycans on the surface of a harmless *E. coli* strain capable of surviving in the gut (reviewed in ref. 2). This has potential cost advantages over the complex synthetic anti-infectives referred to above, because the bacteria can be produced cheaply by large-scale fermentation. A range of receptor mimic probiotics has been constructed by manipulation of the outer core region of the lipopolysaccharide (LPS) through expression of cloned heterologous glycosyltransferase genes. These were sourced from the LPS core regions of other Gram-negative bacteria known to express molecular mimics of specific host oligosaccharide structures on their surface. The bacteria concerned include mucosal pathogens, which presumably mimic the host structures to evade detection by the immune system.

Receptor Mimic Probiotics for STEC Infections

The prototype receptor mimic probiotic was designed to neutralize Shiga toxin (Stx), an AB₅ toxin which is produced by all STEC strains, as well as *Shigella dysenteriae* type 1. STEC is a broad *E. coli* pathotype that includes the notorious O157:H7 serotype.¹¹ It is an important cause of gastrointestinal disease in humans, particularly since such infection may result in haemorrhagic

colitis (HC) and the life-threatening haemolytic uraemic syndrome (HUS), defined as a triad of microangiopathic haemolytic anaemia, thrombocytopenia and renal failure. The pathology of severe STEC disease has long been considered to be directly attributable to the production of Stx. Pathogenesis of human disease initially involves colonization of the gut by STEC. Locally produced Stx is translocated across the intestinal barrier and absorbed into the circulation. The toxin then targets specific tissues in accordance with their expression of its specific receptor, the glycolipid globotriaosyl-(Gal α 1-4Gal β 1-4Glc-) ceramide (Gb₃). In humans Gb₃ is found in highest concentrations in renal tubular epithelial cells and in microvascular endothelial cells, particularly in the kidneys, gut and brain, and Stx-mediated damage at these sites is consistent with the pathology of HUS. Microvascular and concomitant ischaemic damage to the intestinal wall also accounts for the severe bloody diarrhea associated with HC.¹¹

We initially chose a derivative of *E. coli* R1 (CWG308) as the host for expression of a Gb₃ receptor mimic, as it has a *waaO* mutation which results in truncation of its LPS, such that it comprises just the lipid A and inner oligosaccharide core components, terminating in glucose (Glc).¹² We then introduced two *Neisseria*

galactosyltransferase genes (*lgtC* and *lgtE*) on a low copy plasmid (pK184). For *lgtC*, this also necessitated site-directed mutagenesis to stabilize a known phase-variable poly-G tract within the coding region. The exogenous transferases then directed addition of Gal α 1-4Gal β 1-4 to the terminal Glc acceptor displayed on the truncated LPS of CWG308, generating a chimeric LPS terminating in an exact mimic of the Stx receptor.¹³ The chimeric LPS was incorporated into the *E. coli* outer membrane and presented as a high-density array of receptors, capable of high-affinity binding to Stx (Fig. 1A). For both major Stx types (Stx1 and Stx2) one mg dry weight of recombinant bacteria could neutralize over 150 μ g of purified toxin.¹³ The high binding capacity is probably a consequence of the density of receptor mimics displayed on the bacterial surface, as well as the ability of the LPS to diffuse laterally in the outer membrane, allowing optimal docking between each toxin B subunit and its cognate receptor. Twice daily administration of the receptor-mimic bacteria to mice infected with highly virulent STEC strains was 100% effective at preventing otherwise fatal disease.¹³ We have also shown that the mimics are efficacious even when administered in killed form, although it was necessary to increase the frequency of administration to three times per day in order to maintain 100% protection.¹⁴ This was attributed to more rapid clearance of the killed mimic from the mouse GI tract. The killed construct is very stable and maintains toxin-binding capacity for more than a year in liquid form at 4°C, and potentially much longer if lyophilized. Killed recombinant bacteria are no longer

classified as GMOs and might have a smoother regulatory path, and better acceptance by consumers.

As a prelude to human trials, we have further refined the first generation probiotic by developing a nutritional selection system, which obviates the need to use antibiotics during production, and ensures plasmid stability and receptor mimic expression *in vivo*.¹⁵ This system also uses a host strain derived from *E. coli* K-12, which was subjected to comprehensive human safety trials in the early 1980s. First, we deleted both the *waaO* and *waaB* genes from the *E. coli* K-12 strain C600, such that it has the same LPS core structure as CWG308. We then deleted the *thyA* gene from this strain, rendering it thymine-dependent. Meanwhile, we deleted the kanamycin resistance gene from pK184 and replaced it with *Salmonella thyA*. The stabilized *Neisseria lgtC* gene along with *lgtE* were then cloned into this plasmid and transformed into the C600 $\Delta waaOB \Delta thyA$ host. The plasmid was stably maintained, and the construct produced a modified LPS and neutralized both Stx1 and Stx2. Moreover, like the prototype Gb₃ mimic, it fully protected mice against otherwise fatal challenge with highly virulent STEC strains.¹⁵

Receptor mimic probiotics are likely to be most effective when administered early in the course of disease, preferably prophylactically. In humans, the severe Stx-mediated systemic complications of STEC disease usually occur 7–10 days after onset of gastrointestinal symptoms. Thus, there is a clear window of opportunity to deploy a recombinant probiotic to limit disease progression by blocking further uptake of toxin from the gut lumen. Although the progression of STEC disease in the mouse model is compressed relative to human disease, we were able to delay administration of the probiotic for up to 48 hours after STEC challenge and still achieve significant protection.¹⁴ In the clinical setting, optimal deployment of receptor mimic probiotics would be dependent on rapid diagnosis of STEC infection, using ELISA or PCR screening assays to test patient stool samples.^{16,17} However, persons deemed to be at high risk of STEC infection, such as close contacts of confirmed cases or individuals known to have been exposed to a suspected contaminated food source, could be readily identified and treated prophylactically.

A distinct subgroup of STEC strains are known to cause oedema disease, a frequently fatal illness in weanling piglets. These strains produce a variant toxin Stx2e, which unlike the other members of the Stx family, binds to globotetraosyl ceramide (Gb₄; GalNAc β 1-3Gal α 1-4Gal β 1-4Glc-ceramide) preferentially over Gb₃.¹⁸ We modified the Gb₃-mimic bacterium such that it mimics Gb₄ by introducing an additional *N*-acetylgalactosaminyltransferase gene (*lgtD* from *N. gonorrhoeae*). We also introduced a UDP-*N*-acetylglucosamine-4-epimerase gene (*gne* from *E. coli* O113), because CWG308 is unable to produce the activated sugar precursor UDP-GalNAc. As expected, the Gb₄-mimic bacterium had a reduced capacity to neutralize Stx1 and Stx2c *in vitro*, but neutralized 98.4% of the cytotoxicity in crude Stx2e extracts.¹⁹ Piglets are most at risk of oedema disease immediately after weaning, and so routine supplementation of the weaning diet with the Gb₄-mimic probiotic would be a potentially cost-effective prophylactic.

Recently, a subset of STEC strains that are of high human virulence in spite of lacking the locus of enterocyte effacement, have been shown to produce an additional AB₅ toxin called subtilise cytotoxin (SubAB).²⁰ This is the prototype of a new family of AB₅ toxins, as its A subunit, SubA, has distinct enzymic activity; it is a subtilase-like serine protease, the only known substrate of which is the essential endoplasmic reticulum (ER) chaperone BiP/GRP78.²¹ Cleavage of BiP by the toxin induces a massive ER stress response that ultimately triggers apoptosis.²² Glycan array analysis indicated that the SubB pentamer is highly specific for glycans terminating in α 2-3-linked *N*-glycolylneuraminic acid. Binding to glycans terminating in α 2-3-linked *N*-acetylneuraminic acid (NeuAc), which differs by a single hydroxyl group, is about 20-fold weaker.²³ Nevertheless, significant *in vitro* neutralization of SubAB cytotoxicity could be achieved using a CWG308 derivative that displays a mimic of the ganglioside GM2 (GM2 contains NeuAc α 2-3-linked to a subterminal Gal).²⁰ Expression of the GM2 mimic required introduction of the *Neisseria* β 1-4galactosyltransferase gene *lgtE*, as well as an α 2-3sialyltransferase (*cstII*), and a β 1-4*N*-acetylgalactosaminyltransferase gene (*cgTA*) from *Campylobacter jejuni* NCTC11168, along with the UDP-GalNAc-4-epimerase gene (*gne*) from *E. coli* O113 on two separate plasmids.²⁴ Although SubAB is highly lethal when injected into mice, causing massive thrombotic microangiopathy and multiple organ damage,^{20,25} its contribution to human disease is unproven. However, a combination of the Gb₃ and GM2 mimic probiotics is likely to be effective against STEC strains that produce both Stx and SubAB.

Receptor Mimic Probiotics for ETEC Infections

ETEC is a distinct *E. coli* pathotype, which is also a major cause of diarrheal disease in humans and animals. ETEC disease is endemic in developing countries, and apart from being a major killer of young children, it is a common cause of diarrhea in visitors to these regions (hence the term “travelers’ diarrhea”).²⁶ ETEC strains produce the so-called labile enterotoxin (LT), an AB₅ toxin structurally related to cholera toxin (Ctx). The mode of action of both toxins involves binding to the ganglioside GM1 on the surface of gut epithelial cells via their pentameric B subunits, followed by internalisation and retrograde transport via the Golgi to the ER. The A subunit is then translocated into the cytosol, where it ADP-ribosylates the host cell G_s protein, resulting in uncontrolled stimulation of adenylate cyclase. This, in turn, interferes with ion transport, thereby causing watery diarrhea.²⁶ Human pathogenic ETEC may also produce a heat stable enterotoxin (STa) in addition to, or instead of, LT. STa binds to the extracellular domain of guanylyl cyclase C (GC-C), thereby activating its cytoplasmic enzymic domain.²⁷ This increases the intracellular concentration of cGMP, which also dysregulates cellular ion transport, resulting in secretory diarrhea.²⁶ The extracellular domain of GC-C is heavily glycosylated, and fucosylated glycolipids found in human milk have been reported to inhibit STa activity.²⁸ However, other studies suggest that these glycans are not absolutely required for binding

of STa, and non-glycosylated GC-C expressed in *E. coli* has been reported to bind STa in vitro.²⁹

Travelers' diarrhea is well-suited to prophylactic use of toxin-binding probiotics, because it has a high strike rate in people from developed countries visiting regions where ETEC are endemic, and moreover, such travel is usually planned well in advance. However, development of an effective LT-binding probiotic is complicated, because the preferred receptor GM1 is also mimicked by certain strains of *Campylobacter jejuni* that have been associated with autoimmune neuropathies in humans, as discussed later. Fortunately, however, the B subunit of LT is also capable of interacting with other oligosaccharide epitopes, including lacto-N-neotetraose (LNT; Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc).^{30,31} We therefore constructed a bacterium expressing a mimic of LNT, by expressing the *N. gonorrhoeae* *lgtA*, *lgtB* and *lgtE* transferase genes in CWG308.²⁴ This construct neutralized approximately 94% of the LT activity in ETEC culture lysates and when tested with purified LT, it adsorbed approximately 5% of its own weight of toxin. Pre-absorption with, or co-administration of the LNT mimic also reduced LT-induced fluid secretion in rabbit ligated ileal loops in vivo.²⁴ A mimic of lactosyl ceramide, constructed by expressing just *lgtE* in CWG308 was even more effective at neutralizing LT (Paton AW, unpublished data). However, an effective anti-ETEC probiotic would also need to cover STa-producing strains. This will necessitate defining the precise epitope on GC-C that is recognized by STa in order to design constructs that mimic either the oligosaccharide or peptide receptor. In addition to their therapeutic potential, distinct receptor mimic constructs capable of neutralizing LT or STa, respectively, would be useful tools to probe the relative importance of each enterotoxin to ETEC diarrhea in humans.

Receptor Mimic Probiotics for Cholera

Cholera is a life-threatening gastrointestinal disease, which is endemic in Asia, and causes epidemics in nearly all parts of the globe.³² The disease generally results from ingestion of water or food (usually undercooked shellfish) contaminated with *Vibrio cholerae*. The organism passes through the stomach, colonizes the small intestine and releases Ctx, which is a sine qua non of virulence. Like LT, the preferred receptor for the Ctx B subunit is the ganglioside GM1, while A subunit-mediated dysregulation of ion transport causes the massive diarrhea and electrolyte imbalance that is the hallmark of cholera. Without treatment, the case fatality rate for severe cholera is approximately 50%.³² However, in situ neutralization of Ctx in the gut should prevent the disease from developing or at least speed recovery from an established infection with *V. cholerae*. Notwithstanding the structural and functional homology between the toxins, the LNT receptor mimic bacterium referred to above neutralized Ctx less efficiently than it did LT.²⁴ We therefore constructed a GM1 mimic by expressing the genes required for expression of the GM2 oligosaccharide structure in CWG308 (*N. gonorrhoeae* *lgtE*, *E. coli* O113 *gne*, and *C. jejuni* NCTC11168 *cstII* and *cgtA*), as described above, along with an additional *C. jejuni* NCTC11168 gene *cgtB*, which encodes a β -1,3 galactosyltransferase.³³ The recombinant

bacterium was capable of binding purified Ctx with high avidity (Fig. 1B), and was capable of adsorbing >5% of its own weight of toxin in vitro. Administration of the GM1-expressing probiotic also protected infant mice against challenge with virulent *V. cholerae*, even when treatment was delayed until after establishment of infection. When treatment commenced 1 h after challenge, 12/12 mice given the probiotic survived, compared with only 1/12 for control mice ($p < 0.00001$). When treatment commenced 4 h after challenge, respective survival rates were 8/12 and 2/12 ($p = 0.036$).³³ These findings are significant in that the mimic was clearly capable of binding Ctx in the gut lumen in direct competition with the natural receptor on the epithelial surface.

The protection observed in the infant mouse cholera model strongly suggests that prophylactic administration to humans at risk of infection, for example during epidemics, is likely to be highly protective. Moreover, the significant protection in the mouse model achieved in spite of up to a 4 h delay in commencement of probiotic therapy also suggests therapeutic potential in symptomatic humans. In such patients, the capacity of the probiotic to adsorb free Ctx may block further toxin-mediated intestinal injury, even when significant numbers of *V. cholerae* are still present in the gut, thereby shortening the illness. Of course, maximal clinical benefit is likely to be achieved if therapy is commenced as soon as a diagnosis of cholera is suspected for a given patient. The GM1-mimic is stable for long periods in either liquid or dried form without refrigeration, and so might be conveniently formulated in sachets with oral rehydration salts, which are the current mainstay of cholera therapy.³²

Application of the recombinant receptor mimic strategy to prevention of cholera is potentially complicated by the theoretical possibility that the construct might elicit an anti-GM1 response in recipients and trigger autoimmune neuropathies, particularly Guillain-Barré syndrome (GBS). This is based on the established association between GBS and antecedent enteric infection with serotypes of *C. jejuni* that express GM1-related LPS structures.³⁴⁻³⁶ However, establishing a direct causal relationship has been complicated by several factors. Firstly, not all GBS-associated *C. jejuni* strains express GM1 mimics, implicating other LPS structures, particularly those with multiple sialic acid groups.³⁵⁻³⁷ Moreover, a small proportion of GBS-associated strains do not express sialated ganglioside mimics at all.³⁸ Secondly, not all *C. jejuni* serotypes known to express GM1-like LPS have been associated with GBS. This suggests that a GM1-like LPS structure, even if required, may not be sufficient for elicitation of an autoimmune response, and that additional bacterial or host factors play an important role.³⁶ Establishing a causal relationship between infection with *C. jejuni* strains expressing particular LPS structures (or carrying genes encoding enzymes that are responsible for synthesis of such structures) with subsequent development of GBS is further complicated by the fact that expression of key genes such as *cgtB* is subject to bi-directional phase variation, which will generate populations of cells expressing multiple LPS types.³⁹⁻⁴¹ Moreover, mutations in *cstII* resulting in single amino acid differences have also been shown to determine the capacity of the encoded sialyltransferase to add sialic acid at either single or multiple positions

in the LPS core structure.^{38,39,41} Immunization of rabbits with GM1-like *C. jejuni* LPS has been reported to elicit anti-GM1 antibodies and flaccid limb weakness in a proportion of animals, but this required subcutaneous immunizations with LPS in the presence of complete Freund's adjuvant every three weeks for up to a year.⁴² Mucosal presentation of a GM1 epitope on the surface of a non-invasive probiotic bacterium would seem much less likely to break immune tolerance. Commensal gut microorganisms are known to express a wide variety of oligosaccharides, many of which mimic human surface receptors, and mere presence of a human antigen is generally not sufficient to invoke autoimmune responses. Indeed, on-going exposure to these "self antigens" may be important in fine-tuning self/non-self immune recognition and maintenance of the tolerant state.¹ Nevertheless, extensive pre-clinical studies will be needed to ensure that oral administration of GM1-mimic probiotics do not elicit inappropriate antibody responses. Clearly, it would be prudent to commence any human trials of GM1-mimic bacteria using killed preparations.

Future Targets for Receptor-Mimic Probiotics

There are, of course, many other significant bacterial, viral and protozoan enteric infections, which might be targeted by appropriate receptor-mimic probiotics. One of the strengths of this approach is that it would not apply selective pressure for evolution of resistance by the targeted pathogen. Interruption of pathogenesis by blocking toxin-mediated host damage would not interfere with the capacity of a pathogen to survive and reproduce in the environment to any significant extent. Also, any mutation in a toxin or adhesin amino acid sequence that prevented binding to a receptor mimic would logically also prevent interactions with the natural target, and in so doing, would compromise capacity to colonize the host and/or cause disease.

A high-priority target for receptor-mimic probiotics is *C. difficile*, which causes hospital-acquired diarrhea and life-threatening pseudomembranous colitis. In the USA alone, there are 250,000 cases, incurring approximately US\$1 billion in additional health care costs each year.⁴³ *C. difficile* produces two large cytotoxins, TcdA and TcdB, which are responsible for the disease symptoms. Both of these act by glycosylating members of the Ras superfamily of small GTPases, thereby disrupting vital signalling pathways in target host cells.⁴³ The relative importance of the two toxins to human disease is uncertain, and although studies using purified toxins suggested that TcdA was more cytotoxic than TcdB in vitro, *C. difficile* strains producing only TcdB have been associated with human disease.⁴³ This is consistent with a recent study using defined *tcdA* and *tcdB* knock-out mutants of *C. difficile*, which demonstrated that TcdB, not TcdA, is critically important for pathogenesis, at least in the hamster model.⁴⁴ *C. difficile* disease is

highly amenable to receptor-mimic probiotic therapy, because of its high prevalence and the fact that the principal at-risk population (hospitalized patients undergoing antibiotic therapy) is easily identifiable and accessible prior to onset of symptoms. However, development of an effective receptor mimic construct will require information on the identity of the TcdA and TcdB receptors on human cells. Cross-linking studies suggest that TcdA recognizes glycoprotein 96 (gp96) on the surface of human colonocytes, and RNA knock-down of gp96 reduces susceptibility to TcdA cytotoxicity.⁴⁵ However, the actual glycans displayed on gp96 are not known. TcdA is known to recognize a variety of oligosaccharide structures, including Gal α 1-3Gal β 1-4Glc β - (which is not present in humans), and various fucosylated blood group antigens (e.g., Lewis^x and Lewis^y) which are known to be present in human intestinal epithelium.^{46,47} Also, recent glycan array data from the Consortium for Functional Glycomics (www.functionalglycomics.org/glycomics/publicdata/selectedScreens.jsp) indicate that the carbohydrate-binding domain of TcdA binds most strongly to glycans terminating in Gal β 1-3[Fuc α 1-4]GlcNAc β 1-3Gal β 1-4-, which mimics the blood group antigen Lewis^x. However, similar screens using TcdB yielded no potential targets among the >300 glycan structures represented on the array.

Receptor mimic probiotics also may have therapeutic potential for non-infectious diseases. For example, some human milk oligosaccharides, e.g., sialyl-Lewis^x, interfere with selectin-ligand interactions and so have anti-inflammatory properties.⁴⁸ Mimicry of the Lewis^x oligosaccharide on the surface of both *H. pylori* and *Schistosoma mansoni* is also thought to downregulate T_{H1} responses during infection with these organisms, thereby suppressing cell-mediated immunity.⁴⁹ Thus, probiotics expressing appropriate oligosaccharide structures have a potentially exciting role in the treatment or prevention of inflammatory conditions of the GI tract.

Unfortunately, progression of receptor-mimic probiotics into the clinic has been hampered by perceived market place resistance to the use of GMOs in general as therapeutic agents. Although regulatory issues can be addressed, or circumvented by the use of killed receptor mimic constructs in the first instance, the commercial sector has been reluctant to embrace the concept. However, the continued threat posed by infectious diseases in the 21st century, combined with the lack of effective vaccines against many important pathogens and increasing problems due to antibiotic resistance, demand a more open-minded approach.

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