Reviews and Opinions

The Role of Carbohydrates in Infection Strategies of Enteric Pathogens

Kentaro Kato1,2* and Akiko Ishiwa1,2
Received 20 September, 2014  Accepted 4 November, 2014  Published online 15 November, 2014

Abstract: Enteric pathogens cause considerable public health concerns worldwide including tropical regions. Here, we review the roles of carbohydrates in the infection strategies of various enteric pathogens including viruses, bacteria and protozoa, which infect the epithelial lining of the human and animal intestine. At host cell entry, enteric viruses, including norovirus, recognize mainly histo-blood group antigens. At the initial step of bacterial infections, carbohydrates also function as receptors for attachment. Here, we describe the function of carbohydrates in infection by *Salmonella enterica* and several bacterial species that produce a variety of fimbrial adhesions. During invasion by enteropathogenic protozoa, apicomplexan parasites utilize sialic acids or sulfated glycans. Carbohydrates serve as receptors for infection by these microbes; however, their usage of carbohydrates varies depending on the microbe. On the surface of the mucosal tissues of the gastrointestinal tract, various carbohydrate moieties are present and play a crucial role in infection, representing the site of infection or route of access for most microbes. During the infection and/or invasion process of the microbes, carbohydrates function as receptors for various microbes, but they can also function as a barrier to infection. One approach to develop effective prophylactic and therapeutic antimicrobial agents is to modify the drug structure. Another approach is to modify the mode of inhibition of infection depending on the individual pathogen by using and mimicking the interactions with carbohydrates. In addition, similarities in mode of infection may also be utilized. Our findings will be useful in the development of new drugs for the treatment of enteric pathogens.

Key words: bacteria, carbohydrate, enteric pathogen, infection, protozoa, virus

INTRODUCTION

Enteric pathogens, many of which are zoonotic, exert a major impact on public health worldwide including tropical regions. In humans and animals, the enteric pathogens, which include viruses, bacteria and protozoa, infect the intestine epithelial lining, resulting in food poisoning or diarrheal disease. When enteric pathogens enter humans or animals via the oral route, they must withstand the proteolytic conditions in the stomach before penetrating the mucus layer and accessing the underlying gut epithelium for attachment or cell invasion. Adhesion of the enteric pathogens to the intestine epithelial tissue is a prerequisite for the initiation of infection. In many systems it is mediated by lectins present on the surface of the pathogen that bind to complementary carbohydrates on the surface of the host cells. Carbohydrates such as heparan sulfate have been reported to play a crucial role in the entry or budding of viruses [1], and bacterial lectins typically act in the form of elongated submicroscopic multisubunit protein appendages, known as pili [2]. Recently, the surface proteins of apicomplexan parasites have also been reported to bind to carbohydrates on host cells [3]. Thus the initial steps of host cell recognition by enteric pathogens may incorporate common strategies.

Once pathogens invade the host cells, they initiate their survival mechanisms to avoid extermination by host immunity. Ultimately, if infection of host cells could be inhibited, proliferation of the pathogens could be prevented and pathogenesis could be controlled. Insights obtained from studies designed to address this concept will be invaluable to develop novel therapies using innovative drug...
design and engineered vaccine candidates to limit the infectivity of widespread enteric pathogens. Here, we review the recent major advances in research on the role of carbohydrates in the infection strategies of enteric pathogenic viruses, bacteria and protozoa. We further discuss how our knowledge regarding these carbohydrates may influence prophylactic and therapeutic drug development for the treatment of diseases caused by enteric pathogens.

**Interaction between Enteric Viruses and Carbohydrates**

Carbohydrates function as receptors for virus entry. Negatively charged carbohydrates, which are expressed on many types of cells and tissues such as sialic acid and heparan sulfate, are common viral receptors. Orthomyxovirus, polyomavirus, reovirus, coronavirus, paramyxovirus and parvovirus recognize sialic acid as a receptor. Adeno-associated virus, herpesvirus and flavivirus recognize heparan sulfate. On the other hand, the enteric virus norovirus recognizes histo-blood group antigens (HBGAs), which are not charged.

Here, we focus on the association of carbohydrates with norovirus as the virus enters the host cell. Norovirus, a member of the family *Caliciviridae*, is a major cause of acute water- and food-borne gastroenteritis [4]. Norovirus infection is associated with up to 90% of epidemic non-bacterial acute gastroenteritis cases worldwide [5]. Noroviruses are divided into at least five genotypes, three of which (genogroups I, II, and IV) infect humans. Except for a few genotypes, all noroviruses bind to HBGAs including ABH antigens and Lewis antigens [5, 6]. In HBGAs, carbohydrate core structures constitute antigenically distinct phenotypes, namely type 1 (Galβ1-3GlcNAcβ) and type 2 (Galβ1-4GlcNAcβ). H antigen (Fuc-α1-2Gal), i.e., O-type antigen, is generated by fucose transfer to a galactose residue with an α1-2 linkage of type 1 or type 2. The A antigen (GalNAc α1-3(Fuc-α1-2)Gal) and B antigen (Gal α1-3(Fuc-α1-2)Gal) of HBGAs are generated by transfer of GalNAc and Gal, respectively, to an H structure irrespective of the carbohydrate core structure. FUT1 and FUT2 are α1,2FUTs that catalyze the transfer of Fuc to the Gal residue of type 1 and 2 chains, thereby resulting in the synthesis of H type 1 and H type 2, respectively. HBGAs are found in saliva and mucosal secretions from the intestinal epithelial cells of secretors (i.e., individuals who have the FUT2 gene that encodes a fucosyltransferase).

Non-secretors, who do not express FUT2 fucosyltransferase and consequently do not express H type 1 or Le* in the gut, are not infected after challenge with the prototype strain of norovirus, NV/68 [5, 6]. Moreover, the association data between blood type and NV/68 infection showed that, among secretor volunteers, blood groups O and A were associated with an increased risk of infection, while blood group B was associated with a decreased risk. On the other hand, epidemiological studies have shown that some norovirus strains with ABH phenotypes that differ from that of NV/68 can infect individuals. GII/4, which is known as a global epidemic strain, binds more HBGAs than other strains, suggesting that the strength of transmission of GII/4 strains is related to the broad recognition of HBGAs [6]. The recognition sites on HBGAs by norovirus

![Fig. 1. Schematic image of virus (in this case norovirus) recognition of carbohydrates on HBGAs at the host cell entry step.](image-url)

- (A) Fucose (Fuc) and N-acetylgalactosamine (GalNAc) on A antigen are recognized by norovirus. (B) Fucose (Fuc) on O antigen is recognized by norovirus. (C) Fucose (Fuc) and galactose (Gal) on B antigen are recognized by norovirus. (D) Fucoses (Fuc) on Le* is recognized by norovirus.
have been classified according to the interaction of the virus with the H, A, B, and Le epitopes (Fig. 1) [6, 7]. HBGAs are important factors for determining host specificity, although it is still unclear whether HBGAs act as the primary receptor or enhance norovirus infectivity. Researchers including ourselves have demonstrated that feline calicivirus (FCV), a member of the genus Vesivirus, infects the upper respiratory tract by attaching to α2-6-linked sialic acids and using junctional adhesion molecule-1 for internalization [8, 9]. It is comparatively easy to study the life cycle of FCV because the virus replicates efficiently in cell culture without specific supplementation, whereas noroviruses are not cultivable in cell culture.

**INTERACTION BETWEEN ENTERIC BACTERIA AND CARBOHYDRATES**

Carbohydrates also function as receptors for bacterial attachment at the initial step of infection. Here, we describe the role of carbohydrates in bacterial infections, focusing on *Salmonella enterica* and several assortative bacterial species that produce a variety of fimbrial adhesions (Fig. 2).

*Salmonella* strains cause disease in diverse mammalian hosts. Some *Salmonella* strains have a narrow host range, such as *Salmonella enterica* serovar Typhi (*S. typhi*) and serovar Paratyphi (*S. paratyphi*), which cause disease only in humans, whereas strains such as *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) and serovar Enteritidis (*S. enteritidis*) cause infection in numerous species including mice, poultry, pigs, sheep, cattle, horses and humans [2].

Infected orally, *Salmonella* reach the intestinal tract and then mainly attach to the M cells of the intestinal epithelium to initiate invasion [10]. After colonization of the intestinal epithelium, Typhoid *Salmonella*, *S. typhi* and *S. paratyphi* can also survive being engulfed by macrophages, which then spread throughout the body via the lymphatic and blood systems. Non-typhoidal *Salmonella*, *S. typhimurium* and *S. enteritidis* cannot survive within macrophages (Fig. 3). They cause gastroenteritis in humans and animals by colonizing the intestinal epithelium and then invading and destroying the M cells and enterocytes [2].

Bacterial adherence requires both specific and non-specific interactions. In the case of *Salmonella*, the negative charge produced by sialic acid on the surface of the host cell is required as a non-specific adherence factor [11]. For their specific interactions, *Salmonella* and assortative bacteria possess various adhesion molecules such as a variety of bacterial fimbriae. At the initial infection step, bacterial attachment is mainly controlled by these bacterial fimbriae. Individual fimbria recognize and bind to specific sugar-containing molecules on the host cell surface. Each bacterial adhesin recognizes a specific structure of its target sugar molecule, and bacterial fimbriae also help to determine the specificity (species, tissue, or cell) of bacterial infection. Two types of representative fimbriae of *Salmonella* and assortative bacteria (Type 1 fimbriae and Type 4 fimbriae) are shown. Type 1 fimbriae are short and highly expressed on the surface of bacteria. Type 4 fimbriae are thin and flexible, expressed at low levels, and are generally located at the polar part of bacteria. FimA and FimH are categorized as Type 1 fimbriae. Long-polar fimbriae (LPF), Plasmid-code fimbriae (PEF) and bundle-forming pili (BFP) are categorized as Type 4 fimbriae. *Salmonella* and assortative bacteria possess various adhesion molecules such as a variety of bacterial fimbriae. At the initial infection step, bacterial attachment is mainly controlled by these bacterial fimbriae. Individual fimbria recognize and bind to specific receptors to promote adhesion to the host cell surface [2, 12].

Long polar fimbriae (LPF) and plasmid-code fimbriae (PEF) are categorized as type 4 fimbriae (Fig. 2). Std fimbriae are categorized as ρ-fimbriae [2, 13–15]. A previous report showed that when one of the fimbriae carried by *Salmonella typhimurium* was deleted, only virulence for mouse was moderately altered, and that multiple fimbrial adhesins were required for full virulence [16]. For *Salmonella* and assortative bacteria, type 1 fimbria is the
Type 1 fimbriae are highly expressed on the bacterial surface, allowing large amounts of bacteria to adhere via the FimH-mannose interaction. The various kinds of type 4 fimbriae play an important role in bacterial infection. Plasmid-encoded fimbria (PEF) is required for bacterial attachment to intestinal epithelial cells. PEF specifically binds to trisaccharide Galβ1-4(Fucα1-3)GlcNAc, also known as the Lewis X (Le^x) blood group antigen [13]. Long polar fimbria (LPF) mediates the adhesion of *S. typhimurium* to murine Peyer’s patches [21]. Extracellular matrix proteins (ECMs) may act as receptors for LPF. ECMs are modified with various carbohydrate moieties, and the presence of mannose inhibits the LPF-ECM interaction. Mannose-containing carbohydrates may participate in bacterial adhesion via LPF [23].

Std fimbriae are categorized as π-fimbriae and are well conserved among *Salmonella* and assortative bacteria but absent from other related bacterial species. Std fimbriae recognize and bind the H type 2 histo-blood group oligosaccharide, the terminal Fucα1-2Galβ1 moiety. *S. typhi* and *S. paratyphi* can survive within the macrophages after they are engulfed by phagocytosis. Non-typhoidal *Salmonella*, *S. typhimurium* and *S. enteritidis*, however, are unable to survive within macrophages.
ates the adhesion of *S. typhimurium* to murine Peyer’s patches [21]. LPF was first described in *S. typhimurium*, and is found in numerous pathogenic *E. coli* strains [22]. Although its specific receptor remains unclear, extracellular matrix proteins (ECMs), which comprise an interlocking mesh of fibrous proteins and glycosaminoglycans, may act as a receptor for LPF of enterohemorrhagic *E. coli* O157:H7 (Fig. 3). ECMs are modified by various carbohydrate moieties, and the addition of mannose inhibits LPF-ECM interaction. Then mannose-containing carbohydrates may participate in bacterial adhesion by LPF [23].

In some cases, type 4 fimbriae are encoded on plasmids. Such plasmids frequently encode virulence factors for host bacteria, and are therefore called “virulence plasmids” [24]. PEF is required for bacterial attachment to intestinal epithelial cells. It specifically binds to trisaccharide Galβ1-4(Fucα1-3)GlcNAc, also known as the Lewis X (Le^a^) blood group antigen (Fig. 3) [13]. The Le^a^ antigen is defined by the presence of Galβ1-4(Fucα1-3)GlcNAc moiety on saccharide chains of glycoproteins or glycosphingolipids; in the human intestine, it is expressed mainly in crypt epithelial cells [25]. *S. typhimurium* possesses PEF as an adhesin that binds to a crypt-specific histo-blood group antigen that may be relevant to the pathogenesis of human infections. Abundant crypt abscesses are commonly found in *S. typhimurium* patients, raising the possibility that the pathogen may bind to human crypt epithelium at a later stage of infection. In a situation where Peyer’s patches are unavailable because of an inflammatory reaction, *Salmonella* can colonize at the crypt epithelium remaining intact and persist on the surface of the host intestinal tract [13, 25].

On the other hand, some type 4 fimbriae participate in “fimbria-mediated (pilus-mediated) conjugal transfer” of so-called “conjugative plasmids”. Conjugative plasmids can also be virulence plasmids if they encode not only the structural genes of the fimbriae but also other virulence factors, such as a drug resistance gene. These conjugative plasmids spread to other bacteria by horizontal transfer, including *C. jejuni* and assortative bacteria, the FimH adhesins of *Salmonella* species shows a high affinity for α-mannosides and a low affinity for aromatic α-mannosides [19]. In the case of *Salmonella*, allelic variation of FimH adhesion directs not only host cell-specific recognition but also distinctive binding to mammalian and avian receptors. This allele-specific binding profile parallels the host specificity of the respective FimH-expressing pathogen [28]. Similarly, the Lewis b (Le^b^) blood group phenotype in combination with secretor status may hinder colonization of *Helicobacter pylori* in certain populations [29]. *H. pylori* express blood group antigen b-binding adhesion (BabA), and BabA binds to Le^b^ antigens. *Salmonella* and assortative bacteria contain various adhesion factors, including several kinds of fimbriae, which contribute to bacterial virulence; however, analyses of their specific receptor moieties and functions are not yet complete [13, 15].

Carbohydrate moieties on the surface of pathogens are also recognized by hosts and trigger host defense mechanisms. The bacterial surface is covered with various kinds of carbohydrates. For gram-negative bacteria, including *Salmonella*, the major carbohydrate component of the bacterial surface is lipopolysaccharide (LPS). LPS is categorized as a glycolipid, and is a major component of the bacterial outer membrane. Because the saccharide moieties of LPS differ structurally from mammalian carbohydrates, they function as targets of the host immune response. To avoid this host immune response, the LPS of some bacteria, for example *C. jejuni*, is structurally similar to the glycosphingolipids of gangliosides [30, 31]. Similarly, the LPS of most *H. pylori* strains expresses the Le^a^ and Le^b^ antigens [29].

Interestingly, the carbohydrate on the surface of the terminal Fucα1-2Galβ1-4GlcNAc moiety. This structure represents the H type 2 oligosaccharide of the O blood group antigen [14]. The H type 2 oligosaccharide of the O blood group antigen moiety is expressed as part of the mucin-type sugar chains of glycoproteins in the host cell. The terminal Fucα1-2 moiety of H type 2 oligosaccharide of the O blood group antigen is essential for the recognition of Std fimbriae (Fig. 3). Carbohydrate molecules act not only as “anchors” for pathogens but also as the determinants of host and tissue specificity. The variety of adhesion factors carried by a bacterium reflects its pathogenic profile, magnitude of virulence, host specificity, and tissue specificity. In the case of *Salmonella* and assortative bacteria, the FimH adhesins show amino acid sequence diversity. This diversity in FimH structure results in the variation in affinity profiles. *E. coli* FimH shows a high affinity for aromatic α-mannosides as well as Manα1-3 structures. On the other hand, the FimH of *Salmonella* species shows a high affinity for α-mannosides and a low affinity for aromatic α-mannosides [19].
host cell itself can be involved in the host defense mechanism. The *Salmonella* flagella component FliC contributes to bacterial attachment to the host cell by interacting with ganglioside molecules on the surface of the host cell, but gangliosides also act as co-receptors for *Salmonella enterica* FliC and promote FliC induction of the human innate immune response [32]. Gangliosides, i.e. sialic acid-containing glycosphingolipids, are ubiquitous components of eukaryotic cell membranes that have been identified as receptors for bacterial toxins and viruses. An *in vitro* assay showed that a nonflagellated mutant of *S. enteritidis*, constructed by disrupting the fliC gene, was about 50-fold less invasive than the wild-type strain, but bacterial adherence was unaffected [33]. At the attachment of *Salmonella enteritidis* FliC to the host cell surface, gangliosides thus function as receptors.

On the other hand, the flagella component protein FliC induces the host innate immune response by binding to Toll-like receptor 5 of the host cell, and gangliosides react as co-receptors with TLR5 on the FliC-induced response. An *in vitro* assay showed that the incorporation of exogenous ganglioside GD1a into the Caco-2 cell membrane increased the effect of FliC. Incubation of Caco-2 cells with a glucosylceramide synthase inhibitor reduced the innate immune response stimulated by FliC [32].

**INTERACTION BETWEEN ENTERIC PROTOZOA AND CARBOHYDRATES**

Human enteropathogenic protozoa include the apicomplexans *Toxoplasma gondii* and *Cryptosporidium* as well as *Giardia* and *Entamoeba histolytica*. They are all zoonotic pathogens that invade and colonize their target tissues in the alimentary tract of the human host. They form hard cysts that resist degradation in the stomach. Host-derived proteases and low pH trigger their excystation [34].

In this section, we describe the role of carbohydrates in *Toxoplasma gondii* invasion of intestinal epithelial cells. The ability of *T. gondii* to infect Chinese hamster ovary (CHO) cells deficient in sialic acids was reduced by 26.9% compared to wild-type cells, indicating that sialic acid is critical for attachment and invasion of *T. gondii* (Fig. 4) [35]. *T. gondii* microneme protein 1 (TgMIC1) forms a macromolecular complex with TgMIC4 and TgMIC6. Single deletion of the TgMIC1 gene significantly decreases the invasion of host cells, suggesting an essential role for TgMIC1 in host cell attachment and invasion of *T. gondii* [36]. Structural analysis of TgMIC1 revealed a novel cell-binding motif called microneme adhesive repeat region (MARR), which provides a specialized structure for glycan discrimination [37]. Carbohydrate microarray analyses showed that TgMIC13, TgMIC1 and its homologue *Neoascus caninum* MIC1 share a preference for α2-3- over α2-6-linked sialyl-N-acetyllactosamine sequences [38]. P104, a PAN/apple domain-containing protein expressed at the apical end of the extracellular parasite, functions as a ligand in the attachment of *T. gondii* to chondroitin sulfate and other receptors on the host cell, facilitating invasion by the parasite (Fig. 4) [39].

*E. histolytica* fibronectin receptor (EhFNR) shows 99% homology to the intermediate subunit-2 of the Gal/GalNAc-specific lectin [44]. Electron microscopy revealed the close association of a purified EhFNR complex to adhesion plates and phagocytic invaginations. Lipid rafts participate in interactions between *E. histolytica* and the host.
extracellular matrix, and it appears that raft-associated Gal/GalNAc lectin serves as a collagen receptor [45].

Cryptosporidium parvum surface receptors, GP900 and proteolytic fragments of the 60-kDa precursor protein, GP40 and GP15, are characterized as mucin-like and heavily O-glycosylated proteins [46–48]. The GP900 and GP40 of sporozoites and merozoites have carbohydrate residues that are bound by αGalNAc-specific lectins, suggesting that αGalNAc residues are involved in the attachment of parasites to host cells via adherence to internal mucus.

Apicomplexan protozoan parasites also induce host innate immune responses via the carbohydrate molecules present on their cell surface [49]. Glycosylphosphatidylinositol (GPI) protein anchors are abundant in the membranes of tachyzoites and other apicomplexan protozoan parasites including Trypanosoma, Leishmania and Plasmodium spp., where they can serve as ligands for innate recognition [50]. The GPI moieties of T. cruzi and P. falciparum were found to be TLR2 ligands [51, 52], and T. gondii both stimulate cytokine production in macrophages and serve as TLR2 as well as TLR4 agonists. In the case of T. gondii, GPI induces TNF-α production in macrophages through the activation of the transcription factor NF-κB [53].

Comparison of the Interactions Between Enteric Microbes and Carbohydrates

Carbohydrates serve as receptors for infections by viruses, bacteria and protozoa, but the usage of carbohydrates by these microbes varies depending on the microbe. At the initial infection step, these organisms do not simply utilize the electrical forces created by the positive and negative charges of the carbohydrates; rather, they make use of other systems in certain instances. One similarity shared by all three microbes regarding their interactions with carbohydrates, however, is that heparan sulfate plays an important role at entry or invasion of the host cell.

Blood group antigen oligosaccharides are highly expressed in the gastrointestinal epithelium [54]. However, there are individual differences in terms of the presence of these antigens. In addition, there are individual differences in sensitivity to pathogens that recognize and bind to blood group antigens, such as norovirus and H. pylori. These individual differences in antigen expression profiles benefit the survival of the host species because the risk of an attack by a fatal virulent pathogen may be decreased to avoid extinction.

The Structure of Carbohydrates on the Surface of the Gastric and Intestinal Epithelium

A large array of glycoproteins, glycolipids and proteoglycans decorate the surface of animal cells. These glycoconjugates mediate many fundamental cellular processes, including cell-cell and cell-matrix adhesion, motility, growth and signaling [55–57]. Mucosal tissues represent the site of infection or route of access for most parasites, including viruses, bacteria and protozoa [58]. On the surface of the mucosal tissues of the gastrointestinal tracts, various carbohydrate moieties are present and play a crucial role in infection.

Mucosal surfaces are coated with a layer of viscous mucus that ranges in thickness from 300 μm in the stomach to 700 μm in the intestine [59–61]. Mucin glycoproteins from mucus-producing cells in the epithelium or submucosal glands are the major macromolecular constituent of mucus and are responsible for the viscous properties of the mucus gel. In addition to forming a relatively impervious gel, which acts as a lubricant, a physical barrier and a trap for microbes, mucus provides a matrix for a rich array of antimicrobial molecules. Underneath the mucus layer, the cells present a dense forest of highly diverse glycoproteins and glycolipids, which form the glyocalyx. Membrane-anchored cell-surface mucin glycoproteins are a major constituent of the glyocalyx in all mucosal tissues. The oligosaccharide moieties of the molecules that form the glyocalyx and the mucus layer are highly diverse, and the average turnover time of the human jejunal glyocalyx is 6–12 h [62]. Consequently, both the secreted and adherent mucosal barriers are constantly renewed and can rapidly adjust to changes in the environment, for example, in response to microbial infection.

Epithelial mucins are a heterogenous family of large complex glycoproteins containing a dense array of O-linked carbohydrates typically comprising over 70% of their mass. The carbohydrate structures present on mucosal surfaces vary according to cell lineage, tissue location and developmental stage [58]. Mucin glycosylation can alter in response to mucosal infection and inflammation, and this may be an important mechanism for unfavorable changes in the niche occupied by mucosal pathogens. The O-linked glycans of mucin proteins contain 1–20 residues, which occur both as linear and branched structures [58].

In addition to the O-linked glycans, mucins contain a smaller number of N-linked oligosaccharides, which have been implicated in folding, oligomerization (MUC2) and surface localization (MUC17) [63–65]. The terminal structures of mucin oligosaccharides are highly heterogeneous.
and vary between and within species as well as between and even within tissues. The array of oligosaccharide structures on individual mucin molecules is also somewhat determined by stochastic events as the mucin protein moves through the Golgi apparatus [66]. The secreted mucins themselves likely function as decoys for adhesins that have been evolved by pathogens to engage the cell surface, as the mucins express many of the oligosaccharide structures found on the cell surface and are constitutively produced in large amounts, constantly washing the mucosal surfaces [58].

Proteoglycans are present on the cell surface [67] and are also components of glycolalyx. Glycosaminoglycan chains are composed of highly sulfated saccharides that give the cell surface a potent negative charge. One of the prototypical membrane proteoglycans is syndecan-1, which carries conserved attachment sites for glycosaminoglycan chains [67]. The syndecans exemplify hybrid proteoglycans because they contain mixtures of the two major types of glycosaminoglycan chains, heparan sulfate and chondroitin sulfate. The other major family of membrane proteoglycans is the glypcans, which contain GPI anchors in a tissue-specific and temporally regulated manner. Their presence in the basolateral membranes of polarized cells varies [68].

Glycolipids are also a component of the cell membrane. A large variety of glycolipids is present on the surface of animal cells. The carbohydrate moieties vary, and each glycolipid may exhibit a special function, as an annular lipid, surface receptor marker or matrix lipid. For brain and neuronal cells, gangliosides (sialic acid-bearing glycolipids) are the major cell surface determinants [69]. Glycolipids function as the receptor for various biologic factors and also as the receptor for various pathogens. They are present at the underneath part of the glycolalyx. Pathogens can recognize the glycolipids, directly bind to the cell membrane, and invade the host cell. Glycolipids also function as receptors for certain effector molecules, such as bacterial toxins, produced by pathogens and directly react with the host cell. For example, cholera toxin binds to ganglioside GM1 [70].

Thus, for pathogens living in the outer mucus layer, it is difficult to make contact with the surface of normal epithelial cells because of the huge amount of mucin that functions as a “decoy” or “physical barrier”. Mucosal pathogens have, therefore, developed mechanisms to subvert these defense mechanisms of the mucosal layer. On the other hand, intestinal M cells, specifically designed to capture and present microbes to the underlying lymphoid tissue, can be regarded as a “hole” in the mucin barrier. The dome epithelium lacks goblet cells and therefore does not produce gel-forming mucins. Their apical cell surface has only sparse microvilli and an apparently thin glycoalyx [71, 72].

M cells are specialized epithelial antigen-transporting cells that constitute a minor proportion (5%~10% in humans and mice) of the follicle-associated epithelium that covers the lymphoid follicles of organized gut-associated lymphoid tissue such as Peyer’s patches [73–76]. Glycoprotein 2 (GP2) was identified as an M cell-specific molecule [77]. The GP2 expressed on M cells functions as a bacterial uptake receptor [77]. GP2 recognizes FimH, a major component of the type 1 fimbriae, which binds to certain glycoproteins on mammalian cells in a mannose-dependent manner [78].

Consequently, even though M cells constitute only a very small percentage of mucosal epithelial cells, they are the major point of attachment and/or entry used by numerous mucosal pathogens including bacteria (e.g., S. typhimurium, Shigella flexneri, Yersinia enterocolitica and Vibrio cholerae), viruses (e.g., reovirus, HIV-1 and polio virus) and parasites (e.g., Cryptosporidia) [72, 79, 80].

**The Association between Carbohydrates and Microbial Infection**

During cell–pathogen interactions (i.e., infection and/or invasion), carbohydrates function as receptors for various pathogens. On the other hand, carbohydrates (glycoconjugates) can also function as a barrier to infection. On the surface of mucosal tissue, the glycoalyx physically prevents microbes from accessing the cell membrane. Some glycoconjugates, a component of the glycoalyx, contain carbohydrate structures that are recognized by pathogens. Mucins often contain oligosaccharide moieties that correspond to the receptor for various pathogens. On the surface of the mucosal layer, microbes binds to these receptor moieties and are captured at the mucus layer, which consequently blocks the infection. Moreover, when secretory mucins containing receptor carbohydrate structures “trap” pathogens, the pathogens are also carried away. M cells are specialized epithelial antigen-transporting cells scattered in the follicle-associated epithelium that covers the gut lymphoid follicles such as Peyer’s patches. M cells can efficiently engulf particles as large as bacteria; however, the mucus layer of M cells and the surrounding area is relatively thin. Glycoconjugates such as GP2 are expressed on the surface of M cells and function as receptors for bacterial attachment [74]. In the case of the host-parasite interaction, the various kinds of glycoconjugates sometimes function as receptors for the invading pathogens, but they can also function as barriers.
and traps for the host defense system.

**Future Perspectives**

In recent years, the damage caused by enteric pathogens, especially norovirus and *Salmonella*, has expanded through the food chain [4, 5, 81]. These pathogens cause food poisoning in humans and gastrointestinal diseases in animals all over the world. Even today, they are often responsible for large-scale outbreaks of food poisoning. Therefore, the prevention and treatment of infections caused by these pathogens is essential.

In this review, we discussed the interaction between host cells and microbes such as viruses, bacteria and protozoa that involve carbohydrates such as sialic acids, heparan sulfate, and the carbohydrate moieties of ABH and Lewis antigens, mannose components, ECMs and Le^x^). The development and use of drugs that target these carbohydrates is anticipated, even though the microbes vary widely and have different modes of infection. Accordingly, when an anti-microbial drug is developed on the basis of the interaction between a microbe and a carbohydrate, host cell modification of the drug’s structure and/or inhibition of the mode of infection will need to be individualized while still taking advantage of the similarities between interactions.

Moreover, the host gastrointestinal tract cell surface, which is the object of microbial infection, is composed of glycoproteins, glycolipids, and proteoglycans. These molecules are potential targets for carbohydrate drugs used in the treatment of infectious diseases.

Oseltamivir and zanamivir are neuraminidase inhibitors that competitively inhibit the activity of the viral neuraminidase on the sialic acid that is found on glycoproteins on the surface of host cells [82]. By blocking the activity of this enzyme, they prevent new viral particles being released from infected cells.

There are various kinds of polysaccharides on the surface of bacteria. Lipoteichoic acid (LTA), a type of glycolipid, is a component of the bacterial cell wall of gram-positive bacteria. Studies have shown that LTA stimulates the immune system [83, 84]. Recently, LTA has been studied for use as a novel kind of biologically active substance.

Recently, sulfated polysaccharides have been analyzed as drug candidates for protozoan infectious diseases [3, 85, 86]. According to our data, the sulfated positions in the carbohydrates can be critical for the inhibitory quality [3]. Collectively, these studies highlight the possibility that carbohydrate drugs may be developed for the prophylaxis and treatment of parasitic infectious diseases. The results of our studies highlight the possibilities for countermeasures against malaria and toxoplasmosis [3, 85].

**Acknowledgments**

This study was supported by Grants-in-Aid for Young Scientists, and Scientific Research on Innovative Areas (3308) from the Ministry of Education, Culture, Science, Sports, and Technology (MEXT) and for Research on global health issues from the Ministry of Health, Labour and Welfare of Japan, the Program for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry (BRAIN), the Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry, The Naito Foundation, and the Program to Disseminate Tenure Tracking System from the Japan Science and Technology Agency (JST). We thank Mr. Tatsuya Iwanaga for his help with the illustrations.

**References**

1. Spear PG, Shieh MT, Herold BC, et al. Heparan sulfate glycosaminoglycans as primary cell surface receptors for herpes simplex virus. Adv Exp Med Biol 1992; 313: 341–353.
2. Ledeboer NA, Frye JG, McClelland M, et al. *Salmonella enterica* serovar Typhimurium requires the Lpf, Pef, and Tafi fimbiae for biofilm formation on HEP-2 tissue culture cells and chicken intestinal epithelium. Infect Immun 2006; 74: 3156–3169.
3. Kobayashi K, Takano R, Takemae H, et al. Analyses of interactions between heparin and the apical surface proteins of *Plasmodium falciparum*. Sci Rep 2013; 3: 3178.
4. Hutson AM, Atmar RL, Estes MK. Norovirus disease: changing epidemiology and host susceptibility factors. Trends Microbiol 2004; 12: 279–287.
5. Lindesmith L, Moc C, Marionneau S, et al. Human susceptibility and resistance to Norwalk virus infection. Nat Med 2003; 9: 548–553.
6. Shirato H, Ogawa S, Ito H, et al. Noroviruses distinguish between type 1 and type 2 histo-blood group antigens for binding. J Virol 2008; 82: 10756–10767.
7. Huang P, Farkas T, Zhong W, et al. Norovirus and histo-blood group antigens: demonstration of a wide spectrum of strain specificities and classification of two major binding groups among multiple binding patterns. J Virol 2005; 79: 6714–6722.
8. Makino A, Shimojima M, Miyazawa T, et al. Junctional adhesion molecule 1 is a functional receptor for feline calicivirus. J Virol 2006; 80: 4482–4490.
9. Stuart AD, Brown TD. Alpha2,6-linked sialic acid acts as a receptor for Feline calicivirus. J Gen Virol 2007; 88: 177–186.
10. Jepson MA, Clark MA. The role of M cells in *Salmonella* infection. Microbes Infect 2001; 3: 1183–1190.
11. Sakarya S, Gokturk C, Ozturk T, et al. Sialic acid is required for nonspecific adherence of *Salmonella enterica* ssp. enterica serovar Typhi on Caco-2 cells. FEMS Immunol Med Microbiol 2010; 58: 330–335.
12. Baumler AJ, Tsolis RM, Heffron F. Contribution of fimbrial operons to attachment to and invasion of epithelial cell lines by *Salmonella typhimurium*. Infect Immun 1996; 64: 1862–1865.

13. Chessa D, Dorsey CW, Winter M, et al. Binding specificity of *Salmonella* plasmid-encoded fimbriae assessed by glycomics. J Biol Chem 2008; 283: 8118–8124.

14. Chessa D, Winter MG, Jakomin M, et al. *Salmonella enterica* serotype Typhimurium Std fimbriae bind terminal alpha(1,2)fucose residues in the cecal mucosa. Mol Microbiol 2009; 71: 864–875.

15. Zhang XL, Tsui IS, Yip CM, et al. *Salmonella enterica* serovar typhi uses type IVB pili to enter human intestinal epithelial cells. Infect Immun 2000; 68: 3067–3073.

16. van der Velden AW, Baumler AJ, Tsolis RM, et al. Multiple fimbrial adhesins are required for full virulence of *Salmonella typhimurium* in mice. Infect Immun 1998; 66: 2803–2808.

17. Misselwitz B, Kreibich SK, Rout S, et al. *Salmonella enterica* serovar typhi adheres to HeLa cells via Fim-mediated reversible adhesion and irreversible type three secretion system 1-mediated docking. Infect Immun 2011; 79: 330–341.

18. Kroglfelt KA, Bergmans H, Klemm P. Direct evidence that the FimH protein is the mannose-specific adhesin of *Escherichia coli* type 1 fimbriae. Infect Immun 1990; 58: 1995–1998.

19. Sharon N. Carbohydrates as future anti-adhesion drugs for infectious diseases. Biochim Biophys Acta 2006; 1760: 527–537.

20. Hinks S, Frankel G, Kaper JB, et al. Role of intimin and bundle-forming pili in enteropathogenic *Escherichia coli* adhesion to pediatric intestinal tissue in vitro. Infect Immun 1998; 66: 1570–1578.

21. Baumler AJ, Tsolis RM, Heffron F. The lpf fimbrial operon mediates adhesion of *Salmonella typhimurium* to murine Peyer’s patches. Proc Natl Acad Sci U S A 1996; 93: 279–283.

22. Bardiau M, Szalo M, Mainil JG. Initial adherence of *EPEC*, *EHEC* and VTEC to host cells. Vet Res 2010; 41: 57.

23. Farfan MJ, Cantero L, Vidal R, et al. Long polar fimbriae of enterohemorrhagic *Escherichia coli* O157:H7 bind to extracellular matrix proteins. Infect Immun 2011; 79: 3744–3750.

24. Johnson TJ, Nolan LK. Pathogenomics of the virulence plasmids of *Escherichia coli*. Microbiol Mol Biol Rev 2009; 73: 750–774.

25. Cooling LC, DS, Koerner TAW. Lewis X and Sialyl Lewis X Glycosphingolipids. Trends Glycosci Glycotechnol 1997; 9: 191–209.

26. Ishiwa A, Komano T. Thin pilus PilV adhesins of plasmid R64 recognize specific structures of the lipopolysaccharide molecules of recipient cells. J Bacteriol 2003; 185: 5192–5199.

27. Ishiwa A, Komano T. PilV adhesins of plasmid R64 thin pili specifically bind to the lipopolysaccharides of recipient cells. J Mol Biol 2004; 343: 615–625.

28. Guo A, Cao S, Tu L, et al. FimH alleles direct preferential binding of *Salmonella* to distinct mammalian cells or to avian cells. Microbiology 2009; 155: 1623–1633.

29. Rothenbacher D, Weyermann M, Bode G, et al. Role of Lewis A and Lewis B blood group antigens in *Helicobacter pylori* infection. Helicobacter 2004; 9: 324–329.

30. Aspinall GO, McDonald AG, Raju TS, et al. Chemical structures of the core regions of *Campylobacter jejuni* serotypes O:1, O:4, O:23, and O:36 lipopolysaccharides. Eur J Biochem 1993; 216: 880.

31. Wirth HP, Yang M, Karita M, et al. Expression of the human cell surface glycoconjugates Lewis a and Lewis y by *Helicobacter pylori* isolates is related to cagA status. Infect Immun 1996; 64: 4598–4605.

32. Ogushi K, Wada A, Niidome T, et al. Gangliosides act as co-receptors for *Salmonella enteritidis* FliC and promote FliC induction of human beta-defensin-2 expression in Caco-2 cells. J Biol Chem 2004; 279: 12213–12219.

33. Van Asten FJ, Hendriks HG, Koninkx JF, et al. Inactivation of the flagellin gene of *Salmonella enteritidis* serotype enteritidis strongly reduces invasion into differentiated Caco-2 cells. FEMS Microbiol Lett 2000; 185: 175–179.

34. Borowski H, Clode PL, Thompson RC. Active invasion and/or encapsulation? A reappraisal of host-cell parasitism by *Cryptosporidium*. Trends Parasitol 2008; 24: 509–516.

35. Monteiro VG, Soares CP, de Souza W. Host cell surface sialic acid residues are involved on the process of penetration of *Toxoplasma gondii* into mammalian cells. FEMS Microbiol Lett 1998; 164: 323–327.

36. Cerede O, Dubremetz JF, Soete M, et al. Synergistic role of micromenal proteins in *Toxoplasma gondii* virulence. J Exp Med 2005; 201: 453–463.

37. Blumenschine TM, Friedrich N, Childs RA, et al. Atomic resolution insight into host cell recognition by *Toxoplasma gondii*. EMBO J 2007; 26: 2808–2820.

38. Friedrich N, Santos JM, Liu Y, et al. Members of a novel protein family containing micromenal adhesive repeat domains act as sialic acid-binding lectins during host cell invasion by apicomplexan parasites. J Biol Chem 2010; 285: 2064–2076.

39. Gong H, Kobayashi K, Sugi T, et al. A novel PAN/apple domain-containing protein from *Toxoplasma gondii* characterizes and receptor identification. PLoS One 2012; 7: e30169.

40. Boothroyd JC, Hehl A, Knoll LJ, et al. The surface of *Toxoplasma*: more and less. Int J Parasitol 1998; 28: 3–9.

41. Dzierzinski F, Mortuaire M, Cesbron-Delauw MF, et al. Targeted disruption of the glycosylphosphatidylinositol-anchored surface antigen SAG3 gene in *Toxoplasma gondii* decreases host cell adhesion and drastically reduces virulence in mice. Mol Microbiol 2000; 37: 574–582.

42. Grimwood J, Smith JE. *Toxoplasma gondii*: the role of parasite surface and secreted proteins in host cell invasion. Int J Parasitol 1996; 26: 169–173.

43. Jacquet A, Coulon L, De Neve J, et al. The surface anti-
54. Camps MA, Azzouz N, et al. Physical, structural, and functional properties of the beta1 integrin-like fibronectin receptor (beta1EhFNR) in Entamoeba histolytica. Infect Genet Evol 2009; 9: 962–970.
55. Mittal K, Welter BH, Temesvari LA. Entamoeba histolytica: lipid rafts are involved in adhesion of trophozoites to host extracellular matrix components. Exp Parasitol 2008; 120: 127–134.
56. Barnes DA, Bonnin A, Huang JX, et al. A novel multidomain mucin-like glycoprotein of Cryptosporidium parvum mediates invasion. Mol Biochem Parasitol 1998; 96: 93–110.
57. Cevallos AM, Bhat N, Verdon R, et al. Mediation of Cryptosporidium parvum infection in vitro by mucin-like glycoproteins defined by a neutralizing monoclonal antibody. Infect Immun 2000; 68: 5167–5175.
58. Strong WB, Gut J, Nelson RG. Cloning and sequence analysis of a highly polymorphic Cryptosporidium parvum gene encoding a 60-kilodalton glycoprotein and characterization of its 15- and 45-kilodalton zoite surface antigen products. Infect Immun 2000; 68: 4117–4134.
59. Yarovinsky F, Sher A. Toll-like receptor recognition of glycosylphosphatidylinositols derived from Plasmodium falciparum. J Immunol 2007; 178: 1791–1797.
1999; 11: 157–163.

76. Kato T, Owen RL. Structure and function of intestinal mucosal epithelium. In: Mestecky J, Lamm ME, McGhee JR, et al., eds. Mucosal Immunology. Amsterdam: Elsevier; 2005.

77. Hase K, Kawano K, Nochi T, et al. Uptake through glycoprotein 2 of FimH(+) bacteria by M cells initiates mucosal immune response. Nature 2009; 462: 226–230.

78. Pizarro-Cerda J, Cossart P. Bacterial adhesion and entry into host cells. Cell 2006; 124: 715–727.

79. Jones B, Pascopella L, Falkow S. Entry of microbes into the host: using M cells to break the mucosal barrier. Curr Immunol 1995; 7: 474–478.

80. Vázquez-Torres A, Fang FC. Cellular routes of invasion by enteropathogens. Curr Opin Microbiol 2000; 3: 54–59.

81. Fatica MK, Schneider KR. Salmonella and produce: survival in the plant environment and implications in food safety. Virulence 2011; 2: 573–579.

82. Burnham AJ, Baranovich T, Govorkova EA. Neuraminidase inhibitors for influenza B virus infection: efficacy and resistance. Antiviral Res 2013; 100: 520–534.

83. de Vos WM. Lipoteichoic acid in lactobacilli: D-alanine makes the difference. Proc Natl Acad Sci U S A 2005; 102: 10763–10764.

84. Morath S, Geyer A, Hartung T. Structure-function relationship of cytokine induction by lipoteichoic acid from Staphylococcus aureus. J Exp Med 2001; 193: 393–397.

85. Ishiwa A, Kobayashi K, Takemae H, et al. Effects of dextran sulfates on the acute infection and growth stages of Toxoplasma gondii. Parasitol Res 2013; 112: 4169–4176.

86. Recuenco FC, Kobayashi K, Ishiwa A, et al. Gellan sulfate inhibits Plasmodium falciparum growth and invasion of red blood cells in vitro. Sci Rep 2014; 4: 4723.