Histo-blood group antigens as mediators of infections

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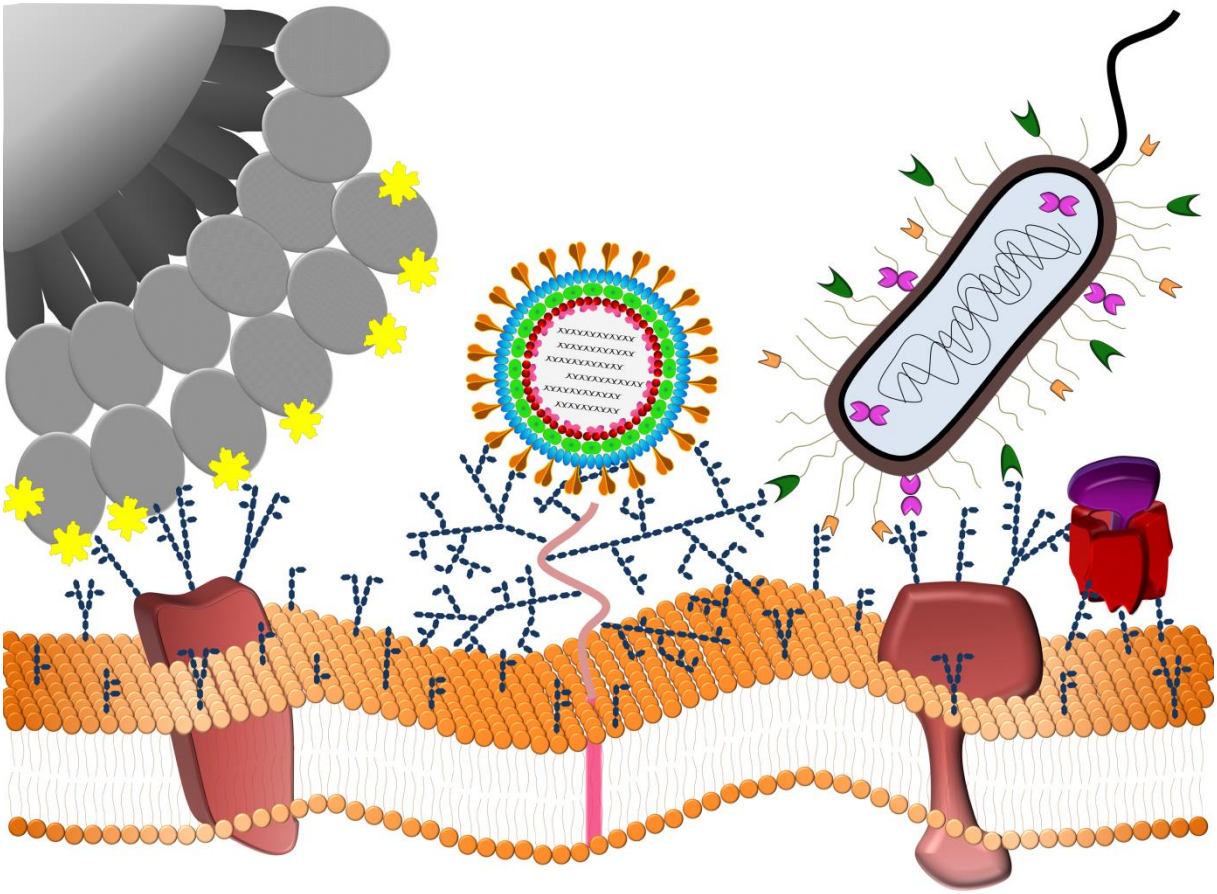

Highlights

- Cells are covered by a dense, protective glycan layer, the glycocalyx
- Viruses, bacteria and fungi exploit these glycans to gain entry to the host
- The mucus layer covering host cell epithelia sheds invaders
- Milk oligosaccharides can serve as protective decoys
- This review describes the structural mechanisms of this molecular arms race

Abstract

The critical first step of a microbial infection is usually the attachment of pathogens to host cell glycans. Targets on host tissues are in particular the histo-blood group antigens, which are present in rich diversity in the mucus layer and on the underlying mucosa. Recent structural and functional studies have revealed significant new insight into the molecular mechanisms, explaining why individuals with certain blood groups are at increased risk of some infections. The most prominent example of blood-group-associated diseases is cholera, caused by infection with *Vibrio cholerae*. Many other microbial pathogens, *e.g. Pseudomonas aeruginosa* infecting the airways, and enterotoxigenic *Escherichia coli* (ETEC) causing traveler's diarrhea, also bind to histo-blood group antigens, but show a less clear correlation with blood group phenotype. Yet other pathogens, *e.g.* norovirus and *Helicobacter pylori*, recognize HBGAs differently depending on the strain. In all cases, milk oligosaccharides can aid the hosts' defenses, acting as natural receptor decoys, and anti-infectious therapy can be designed along similar strategies. In this review, we focus on important infections of humans, but the molecular mechanisms are of general relevance to a broad range of microbial infections of humans and animals.

Introduction

The battle between pathogens and their hosts is a constant race of evolution and adaptation. Once inside the host, either through the airways or the gastrointestinal tract, the pathogens must fight their way to the underlying cells. The mucus layer provides the first protective barrier of the underlying tissues (reviewed by [1]), and is essentially kept sterile by the constant secretion of mucus from goblet cells and the shedding of its top layer. This is true in particular for the colon, which has a second, inner mucus layer that is impermeable to bacteria. The mucus consists of a network of heavily glycosylated proteins, containing negatively charged carbohydrates and a significant amount of neutral, fucosylated sugars. The underlying mucous membrane, the mucosa, contains glycoconjugates including both lipids and proteins, resulting in a dense layer of glycans called the glycocalyx. This layer serves as a protective layer, helping to keep the pathogens at bay. However, many pathogens have evolved an ability to take advantage of these glycans, and use them as receptors for host cell adhesion or entry [2,3].

Especially well-characterized carbohydrate-based antigens are the histo-blood group antigens (HBGAs), which include the ABH and Lewis antigens. The ABH blood group system is named after the expression of antigens by red blood cells. HBGAs are also present on epithelial and endothelial cells throughout the body, and as soluble oligosaccharides in most body fluids except cerebrospinal fluid. Many pathogens use these antigens to adhere to host cells, and the interplay between pathogens and HBGAs is believed to promote the antigen diversity we see today [4,5*]. Individuals have different active glycosyltransferases, leading to a large variety of oligosaccharides, whose expression varies greatly between tissues and even between tissue parts. A rich display of HBGAs is found in particular in the gastrointestinal tract and in the mucus layer covering it. This variation provides an advantage against pathogens, which rapidly evolve to maintain their competitive edge.

HBGAs are synthesized from precursor antigens and modified by the actions of different glycosyltransferases (Figure 1; reviewed by [4]). The simplest ABH antigens are the H antigens characteristic of blood group O, which can be converted into A or B antigens by the action of the A or B glycosyltransferases, respectively. ABH and precursor antigens can be fucosylated on GlcNAc, creating the *Lewis* antigens. The fucosyltransferase Secretor/FUT2 can add the so-called *Secretor* fucose. This enzyme is lacking in 20% of the European and North American population, resulting in the so-called non-secretor phenotype characterized by the absence of all ABH antigens from mucus and secretions like saliva and human milk. Nevertheless, non-secretors can still express the simplest fucosylated Lewis antigens. In addition, symbiotic host microbes are known to contribute to intestinal fucosylation [6*].

The severity and susceptibility of many infectious diseases correlate with blood group phenotype [7]. So far, the understanding of the molecular mechanisms underlying this phenomenon has been very limited. This has changed recently with the determination of several high-resolution crystal structures of relevant protein-HBGA complexes. The gained insights are the topic of this review.

Infections of the gastrointestinal tract

Infection by enteric pathogens generally occurs through the fecal-oral route, caused by poor hygiene, consumption of contaminated food or water, or by exposure to infectious aerosols

that are produced by vomiting. Some pathogens, such as *Helicobacter pylori*, are in addition transmitted through the oral-oral route, and others, *e.g. Vibrio cholerae*, can also survive in aquatic reservoirs, without any contact with feces.

Viral infections

Noroviruses (NoVs) and rotaviruses (RVs) are the two most important causes of acute gastroenteritis in humans. Both of them are 'non-enveloped' viruses, which means that they are encased by a protein capsid lacking a lipid envelope. Viruses replicate and evolve very rapidly, giving rise to a large number of different strains with different receptor profiles that enable them to conquer different niches (reviewed by [8*,9*,10,11*]). Among their known cellular receptors are sialylated structures and HBGAs. Secretors are particularly susceptible to NoV and RV infections [12]. These individuals express HBGAs in their body fluids, mucus and gastrointestinal epithelial cells, therefore it is plausible that they are at higher risk of infection. While many children in the world are routinely vaccinated against RV infections, there is currently no vaccine against NoV infection.

The first examples of human pathogens, for which the molecular basis of blood group dependence was elucidated, are NoVs [13,14*]. They are single-stranded RNA viruses belonging to the *Caliciviridae*. Both major NoV genogroups, GI and GII, are known to infect humans and bind HBGAs in the high micromolar range [15]. GI includes the well-known Norwalk virus, causing winter-vomiting disease. This genogroup mainly targets individuals with blood group O, while people with blood group B have a lower risk of infection [16]. In contrast, GII NoVs do not discriminate between different blood groups [17,18]. The first X-ray structure of a NoV was published in 1999 (PDB ID 1IHM [19]*). Its viral capsid contains dimeric P domains, which project out from the icosahedral shells and are responsible for the binding to the host cell receptors (Figure 2A,B). Intriguingly, the P domains (and in particular the receptor-binding P2 subdomains) have a much lower sequence identity than the overall genomes of GI and GII NoVs (approximately 25% (P2) compared to 50% overall [11*,13]). Moreover, the receptor binding sites are positioned at different locations at the P dimerization interface and have distinct structural characteristics [14*,8*,10] (Figure 2B-D). GI viruses bind HBGAs end-on and recognize mainly the terminal β-Gal residue of blood group H-antigens (Figure 2C). Additional interactions are found to the *Secretor* fucose, which include hydrophobic interactions to a conserved tryptophan residue, kept in place by cation-

π stacking to a histidine. In A or B antigens, the β-Gal binding site is instead occupied by the terminal α-GalNAc or Gal residues characteristic for blood groups A and B, respectively, whereas the fucose residue is reoriented away from the conserved tryptophan into a secondary binding pocket (Figure 2C). In A antigens, the *N*-acetyl group of the terminal α-GalNAc residue mimics the interactions of the *Secretor* fucose (Figure 2C), whereas α-Gal, characteristic of B antigens, lacks these additional interactions and binds poorly to GI NoVs, explaining why individuals with blood group B experience protection. GII NoVs mainly recognize α-fucose, present either as *Secretor* or *Lewis* fucose in a large variety of HBGAs [20-25] (Figure 2D). It is therefore not surprising that GII NoVs exhibit a broad ABH blood group profile.

Significantly less is known about RVs, both in terms of epidemiology and molecular mechanisms. RVs are double-stranded RNA viruses of the *Reoviridae* family, which form much larger viral particles than NoVs. VP8*, a subunit of the outer capsid protein VP4, is the functional equivalent of NoV's P domain (Figure 2E,F). VP8* has a galectin fold, a fold known to recognize β-Gal. However, in RVs the galactose binding site is blocked and a different site is involved in receptor recognition. For several decades, sialic acid has been known as the key cellular receptor of RVs, whereas the binding of HBGAs to certain RV strains was discovered only recently [26,27]. Nevertheless, the limited data that exist suggest that secretors are significantly more susceptible to RV infections than non-secretors [12], indicating that HBGAs may indeed be functional RV receptors. In 2012, a crystal structure of VP8* was reported in complex with a blood group A-trisaccharide [28*], occupying the same site that binds sialic acid in other RV strains. The major interactions were to the α-GalNAc characteristic of blood group A and additional interactions to β-Gal, whereas the *Secretor*-fucose faced away from the HBGA binding site (Figure 2F). Nevertheless, glycan array data showed that this residue strongly enhances receptor binding [28*], which may be due to intramolecular conformational stabilization. Overall, binding specificity varies greatly between different RV strains, and even strains binding the same antigens exhibit considerable variation in molecular recognition [26,27,29]. Differences in the width of the receptor cleft may play a role in the different binding properties [10]. The structural adaptability of the VP8* binding site, including receptor-induced conformational changes,

may furthermore lie at the heart of receptor-release prior to cell entry [22*] and of interspecies transmission [27,30].

Bacterial infections

Many bacteria use lectins to attach to host cells (reviewed in this issue by Moonens & Remaut [31*]). One notable example is *Helicobacter pylori*, responsible for inducing stomach ulcers and gastric cancer. More than half of the world population is infected with this bacterium, which was declared a class I carcinogen by the WHO in 1994. The most virulent *H. pylori* strains mediate adhesion to the human host *via* the Blood group Antigen Binding Adhesin, BabA [32], which is present in large amounts in *H. pylori* membranes and facilitates the bacterial colonization of the stomach mucosa. Different *H. pylori* strains exhibit distinct blood group profiles [33]: in populations with high incidence of the blood group O phenotype, such as the natives of South America, specialist species predominate, while in Europe and the USA, which have a more diverse blood group pattern, generalist species of *H. pylori* are able to bind all three ABH blood group antigens. The different binding profiles are correlated with the presence of BabA variants with different specificities for ABH/Lewis glycans. Moonens *et al.* recently solved the crystal structures of several of these BabA variants in complexes with various HBGAs, revealing the molecular basis of this phenomenon [34**]. BabA's interaction with host receptors is three-pronged (Figure 3A): (i) Its main anchor is the *Secretor* fucose, which is bound by a loop stabilized by a redox-sensitive disulfide bond called Cys-clasped Loop CL2, involving hydrogen bonds to several highly conserved amino acid residues. ii) The Asp-Ser-Ser triad of the Diversification Loop DL2 specifically binds type 1 glycan receptors, ensuring tropism for the foveolar epithelium, which covers the inside of the stomach. iii) Blood group A/B-specific α-GalNAc/Gal residues bind to a shallow pocket in the DL1 region. O-specific *H. pylori* strains exhibit a distinct sequence variation in this loop, where a proline in combination with a bulky residue (such as Asn, Asp, or Leu) sterically interferes with generalist binding. Additional phenotypes are the *AB specialists*, which bind more tightly to A/B-specific glycans due to additional interactions of the terminal α-GalNAc/Gal residues with DL1, and the *inverse specialists*, which bind A/B- rather than O-specific glycans despite exhibiting the bulky Pro-Leu sequence, since a non-functional DL2 loop allows for a slight rotation that prevents the steric clash (Figure 3A).

Cholera is arguably the most well-known example of blood-group-dependent diseases. For 50 years, individuals with blood group O have been known to experience more severe symptoms than those with other blood groups [35], although paradoxically they are less likely to be infected. The main culprit is the cholera toxin (CT), which after gaining entry to host cells, hijacks the host's own endogenous pathways to trigger the opening of ion channels, inducing the massive secretory diarrhea typical of the disease. Secretors experience protection from cholera [36], which is in contrast to many other diseases. In the past few years, great strides were made in understanding the blood group association of cholera on a detailed molecular level. The primary receptor of the CT is the GM1 ganglioside, which binds to the toxin with nanomolar affinity [37]. That CT can also bind to HBGAs or analogs of these has been shown only recently [38,39]. Studies of chimera of the receptor-binding subunits of CT and the homologous heat-labile toxin (LT) from enterotoxigenic *Escherichia coli* (ETEC) indicated that the toxins may harbor a second binding site [40,41], for HBGAs, that is spatially distinct from the GM1 binding site (Figure 3B). Subsequently, the crystal structure of LT was determined in complex with a blood-group-A-specific human milk oligosaccharide (HMO) resembling A-Lewis^y, sparking a discussion as to why ETEC infections nevertheless do not show a strong blood group association [42*]. This is probably due to interference with LPS: in contrast to the CT, which is secreted as a soluble toxin, LT remains attached to outer membrane vesicles *via* LPS. Indications are that the binding sites for HBGAs and LPS overlap, interfering with LT binding to HBGAs [43], which would explain the lack of blood group association for ETEC infections. For cholera, conflicting data were published regarding the binding of two natural CT variants to HBGAs and HMO analogs [38,39,44]. The breakthrough came last year with the high-resolution structures of respective CT-complexes [45**] (Figure 3B) and supporting cell biological experiments [46], explaining all previous findings. Unexpectedly the blood group A determinant (and its HMO analog) bound in the opposite orientation compared to LT, showing that subtle amino acid differences in the secondary binding site can considerably modulate binding. Another unexpected result was the binding of H-determinants (Lewis^y and its HMO-analog) in two alternative orientations. This rationalized the stronger binding of O-specific glycans compared to A- and B-determinants, where the A/B-specific α-GalNAc/Gal residues can only be accommodated in one of the orientations. Again a fucose residue (generally the *Lewis* fucose) was shown to play the central role in binding, connecting two adjacent CT subunits.

Even though binding affinities to HBGAs are only in the millimolar range and hence significantly weaker than to GM1 [37,45**], CT is capable of cooperatively binding these ligands with all five of its receptor-binding subunits, permitting high-avidity interaction with its target tissue. Stronger binding of the toxin to O-specific compared to A/B-specific glycans is consistent with the observed blood group profile of cholera. What remains to be further investigated is why secretors are protected.

Other enteric pathogens known to bind HBGAs are *Campylobacter jejuni* [47] and *Salmonella enterica* serotype Typhimurium [48], although structural data regarding these interactions are not yet available.

Infections of the airways

While healthy airway mucus helps to expel microorganisms by coughing and sneezing, pathogenic mucus, as encountered in cystic fibrosis or chronic obstructive pneumonia, becomes a niche for chronic infection, leading to morbidity and mortality. The glycosylation of mucus changes during the course of pathologies, although it is not yet clear whether the changes are due to the disease itself, to associated inflammation or due to the presence of microbes [49-51]. Modifications in fucosylation, sulfatation and sialylation are generally observed in cystic fibrosis, but with different trends depending on *N*- or *O*-glycosylation. Opportunistic pathogens, often associated with hospital environments, include bacteria such as *Pseudomonas aeruginosa* and members of the *Burkholderia* complexes, and airborne fungi such as *Aspergillus fumigatus*. All of these pathogens bind to HBGAs, but so far only *P. aeruginosa* has been investigated for correlation between blood group and host susceptibility.

P. aeruginosa infection is the leading cause of death of cystic fibrosis patients, and also affects immunocompromised patients. Conflicting data exist regarding its possible correlation with blood group phenotype. While the analysis of gene polymorphism in cystic fibrosis patients did not reveal any correlation between severity of *P. aeruginosa* infection and ABH, secretor or Lewis genotypes [52], other studies indicated a stronger susceptibility to *P. aeruginosa* sepsis in children with blood group B [53] and a correlation of external otitis with blood groups A or B [54]. *P. aeruginosa* interacts with host glycans mainly via two

soluble lectins, the α-galactose specific LecA and the fucose-specific LecB, which have both been structurally characterized [55]. The LecA target is likely the αGal1-4Gal disaccharide present on globotriaosylceramide (Gb3) and blood group P^k and P_1 antigens [56]. LecA binding to Gb3 triggers the uptake of the bacterium into airway cells *in vitro* [57]. LecB has a very strong affinity for fucose due to the rare chelation of two calcium ions, and its best-known ligand is the $Lewis^a$ epitope. Two recent studies investigated the variations of LecB genes in more than 200 bacterial strains originating from natural or clinical environments [58,59**]. Sequence analysis revealed two main clusters that could be identified as PAO1-like and PA14-like. The fucose binding site is conserved in all strains, with 13% variations of sequences between PAO1 and P14. Crystal structures and affinity analysis of two variants from PA14 [59**] and PA7 [58] strains confirmed that binding to fucose is conserved, indicating the importance of LecB for these bacteria. Furthermore, multivalent epitope presentation was shown to be critical. For example, the LecB variant from the pathogenic PA14 strain binds with very high affinity (K_d = 25 nM) to a biantennary *N*-glycan presenting two blood group H epitopes, corresponding to an affinity that is 10-fold higher than for isolated H-type 2 oligosaccharide (K_d = 200 nM) [59**].

The fucose-specific six-blade β-propeller fold represents another lectin family identified in lung pathogens, *i.e. B. ambifaria* (BambL) [60] and *A. fumigatus* (AFL or FleA) [61]. The biological role of these lectins is not yet entirely clear. For example, AFL was demonstrated to contribute to inflammation [61], but also stimulated macrophage killing, thus protecting the host [62*]. The 6-blade β-propeller fold is formed by a tandem repeat of one single chain in fungi or by trimerization of a much shorter chain in bacteria (Figure 4A,B). In AFL, the six binding sites exhibit minor differences in amino acid composition with variations in oligosaccharide preference as observed by soaking of crystals in a mixture of oligosaccharides [63*]. Nevertheless, both bacterial and fungal family members bind fucose in a unique conserved binding mode involving hydrogen bonds between an arginine and the O4 hydroxyl or ring oxygens, and between a Glu/Gln residue and the O3 and O4 hydroxyls as well as a CH-π interaction with a Tyr/Trp residue (Figure 4C-F). The general preference for blood group O (H-type 2) and $Lewis^y$ was rationalized through molecular modeling [64], showing that the binding pockets are well oriented for multivalent binding of terminal αFuc1-2Gal epitopes (Figure 4D). Apart for its preference for $Lewis^y$, AFL binds to all

fucosylated oligosaccharides, and therefore to all tissues expressing Lewis and ABH antigens. The αFuc1-3GlcNAc disaccharide present in Lewis$^{x/y}$ is also recognized, and analysis of the crystal complexes led to the surprising observation that the "closed" conformation, observed in solution, solid state and modeling [65], is forced into an "open" conformation when entering the binding sites (Figure 4E-F). A conserved aromatic residue appears to be responsible for guiding the fucose into the binding site by separating the fucose and galactose rings that are stacked together in solution [66**]. Opening of the canonical Lewis^{x} conformation apparently involves the transient distortion of the central *N*-acetyl-glucosamine ring, as shown by crystal structures of another family member, *Ralstonia solanacearum* lectin (RSL), in complexes with Lewis^{x} and sialyl-Lewis^{x}, combined with extensive molecular dynamics simulations [66**].

Fighting back: glycans, glycocompounds and glycomimetics as anti-infectious agents

Anti-infectious strategies can be based on competition with the attachment of pathogens to HBGAs on target tissues. Such protection is provided naturally by human milk, which contains a large variety of fucosylated and sialylated oligosaccharides (HMOs). These soluble oligosaccharides protect newborns from infections in a dual fashion, as anti-microbials and prebiotics [6*,67*]. Interesting examples, where structural information is available, are cholera [44,45**] and norovirus infections [68]. Building on the accumulated structural knowledge of lectin-oligosaccharide interactions, it has been possible to design glycomimetics with increased affinities for lectin receptors, achieving a stronger competition effect [69]. Classical strategies are centered on optimizing the binding energy between protein and carbohydrates, *i.e.* increasing the enthalpy of binding by maximizing the number of hydrogen bonds and/or hydrophobic contacts, and decreasing the entropy contribution by reducing the flexibility of the oligosaccharide. For example, the careful design of the aglycone (=non-sugar) part of a fucose derivative significantly increased the selectivity for its target *B. cenocepacia* BambL [70]. Similarly, a fucopyranoside glycomimetic inhibitor of *A. fumigatus* FleA was shown to inhibit binding and phagocytosis of conidia by macrophages [62*].

Another strategy is to design multivalent ligands that simultaneously bind to several binding sites of a multivalent receptor, resulting in a strong gain in avidity (=apparent multivalent affinity) [71*,72*]. A successful example is a divalent galactoside ligand of *P. aeruginosa* LecA, which was able to lower the cellular invasiveness of the bacteria by up to 90 % by chelating two neighboring receptor binding sites [73]. Other examples are the prevention of human immunodeficiency virus (HIV) transmission by glycodendrimers [74] and the design of multi-valent inhibitors against bacterial toxins [72*]. Based on high-throughput screening of compounds libraries, both *in vitro* and *in silico,* it is also possible to identify non-carbohydrate glycomimetics able to compete in carbohydrate binding sites, as accomplished for noroviruses [75]. Yet another possibility is the design of HBGA-blocking antibodies as therapeutic agents ([76] and references therein).

Conclusions and Perspective

A large variety of human pathogens recognize blood group antigens. These include viruses, bacteria and fungi, which employ viral coat proteins, microbial adhesins, soluble lectins and toxins to invade and conquer their hosts [2]. In this review, we focus on human infections of the gastrointestinal tract and the airways. A common microbial strategy is to use HBGA binding for attachment and entry to the host cells. Secretors, who display these antigens on tissues exposed to the external environment and on their mucus, nurture symbionts [6*] but are also disproportionally affected by infectious diseases (however, as described below, hosts have their own measures to force selective pressures onto microbes, *e.g.* through blood group polymorphism, to prevent the eradication of entire populations [4,5*]). Another microbial strategy is to employ HBGA-binding toxins. Not discussed in this review is the molecular mechanism of parasitical malaria infection, where adhesins facilitate erythrocyte rosetting by binding primarily to blood group A antigens [77], explaining why individuals with blood group A are most severely affected by this disease. This has consequences on a population level, due to natural selection and evolution. The Ganges delta, for example, where both malaria and cholera are endemic, has a unique blood group profile, with the highest percentage of blood group B in the world. Blood group polymorphism is an important asset for surviving in this world [4]. Microbes respond by evolving many strains with different binding properties, but the hosts can fight back. Of particular importance is the continuously renewing mucus layer, which nurtures symbionts and helps expel

pathogens [1,6*]. Another trick is human milk, which mimics HBGAs, serving as non-degradable receptor decoy protecting human infants [67*]. The high fucose-content of human milk is likely of particular importance for the protective effect. Modified oligosaccharides, glycomimetics and dendrimers work in a similar way, with potential as medications and prophylactics [69,72*]. Enveloped viruses like influenza virus and HIV take their host's membrane coats along on their journey to a new victim. This provides them with disguise, but can also elicit immune reactions against foreign blood groups. The molecular arms race is on, and we are now beginning to understand its molecular underpinnings, providing us with new tools to fight disease.

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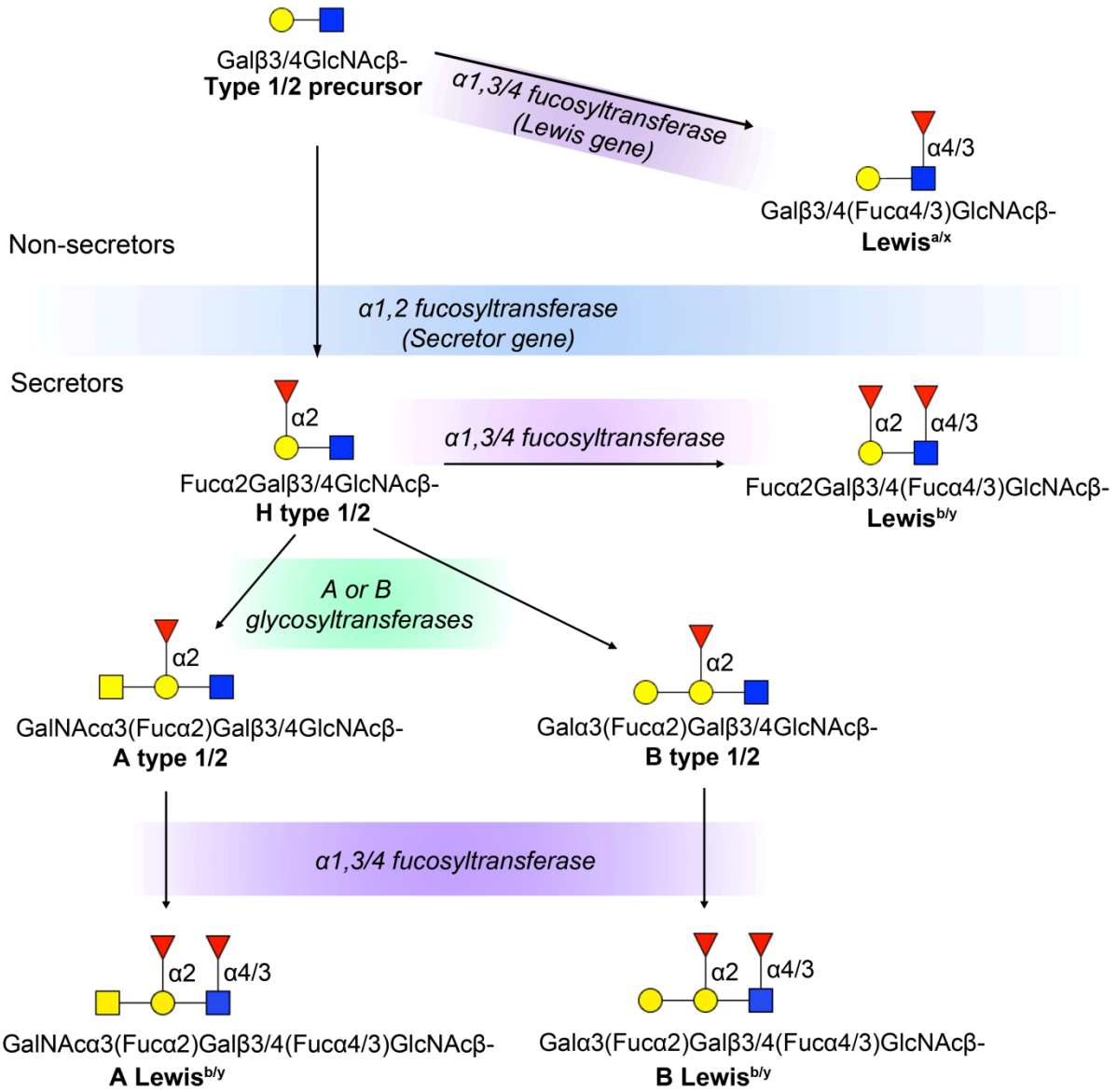
Figure Legends

Figure 1: Biosynthetic pathways of type 1 and type 2 human histo-blood group antigens (HBGAs). Carbohydrate symbols are shown in the standard schematic representation introduced by the Consortium for Functional Glycomics. H-type 1 (α3-linkage) and H-type 2 (α4) precursors are fucosylated by α-1,2-fucosyltransferase (FUT2) or α-1,3/4-fucosyltransferase (FUT3/4), adding the *Secretor* fucose and *Lewis* fucose, respectively. Blood group H determinants (characteristic of blood group O) can subsequently be modified by the action of A or B glycosyltransferases, which add an α-GalNAc or α-Gal residue to β-Gal, to give rise to blood group A or B antigens, respectively. Non-secretors lack the *FUT2* gene and can therefore only synthesize the smallest Lewis antigens, Lewis^a and Lewis^x.

Figure 2: HBGA recognition by noro- and rotaviruses. **(A)** Structure of NoV capsid (PDB 1IHM [19*]). **(B)** Structure of individual protruding P domains (dimers). Gray – P domain of GI NoV in complexes with H-pentasaccharide (green sticks; PDB 2ZL6 [14*]) and A-trisaccharide (cyan sticks; PDB 2ZL7 [14*]). Green/slate – GII P domain bound to A-trisaccharide (yellow sticks; PDB 3SLD [22*]). **(C)** Top view of the GI and GII P domain complexes shown in (B) and close-up view of GI NoV interaction with H-type 1 pentasaccharide (green sticks, with fucose in magenta) and the corresponding A-trisaccharide (cyan sticks, including fucose). Important amino acid and carbohydrate residues are labeled, H-type 1 residues in italics. H-bonds are indicated by red dashed lines, and the hydrophobic interaction to Trp with yellow filled circles. Note that the acetamido group of GalNAc characteristic of A antigens partially mimics the interactions of the H-type 1 fucose. **(D)** Top view of the GII P domain complex shown in (B), and details of GII NoV interaction with A-trisaccharide (yellow sticks, fucose in magenta). The virus is anchored to the fucose residue, while the GalNAc residue provides only limited contacts. **(E)** RV capsid structure (PDB 4V7Q [78*]) with protruding VP4 spikes (teal). **(F)** Top domains of VP4, with A-trisaccharide (yellow sticks, with fucose in magenta) bound to the VP8* domain. The close-up view shows details of VP8* A-trisaccharide interaction (PDB 4DRV [28]). Carbohydrate residues are labeled, the fucose is highlighted in magenta and H-bonds are indicated with red dashes. Main interactions are to the GalNAc residue characteristic of A antigens.

Figure 3: HBGA-interactions of selected bacterial pathogens of the gastrointestinal tract. **(A)** Molecular basis of *H. pylori* adaptation to human HBGA polymorphism. The *H. pylori* adhesin BabA binds to HBGAs with three loops (DL1, CL2, DL2), displaying significant variation between different strains. For example, ABO generalists can accommodate the Gal (green sticks) or GalNAc residues (not shown) characteristic of B or A antigens, respectively, whereas bulky residues in DL1 prevent HBGA binding due to steric interference, leading to O-specialists (close-up view). This panel is reproduced with permission from [31*]. **(B)** Molecular basis of cholera blood group dependence. The culprit is the cholera toxin (CT; cartoon representation), which binds to HBGAs (box) and GM1 (green sticks; PDB 3CHB [79]). Several CT structures are superimposed. The CT A subunit is colored purple (PDB 1LTS [80]), the CT B pentamer blue, gray and magenta (PDB 3CHB [78]). A close-up view shows the detailed interactions of CT with the H-tetrasaccharide (white sticks; PDB 5ELB [45**]) and the A-pentasaccharide (colored sticks; PDB 3ELD [45**]). The color code is the same as for the standard schematic representations shown below. Note that H-tetra can bind in two orientations, whereas the A-pentasaccharide (BGA or HMO) binds in a single orientation (Se Fuc, *Secretor* fucose; Le Fuc, *Lewis* fucose).

Figure 4: HBGA-pathogen interactions of selected airway infections. **(A,B)** Overall representations of the 6-bladed β-propeller folds of BambL in complex with the H-type 2 determinant (**A**, PDB 3ZZV [60]) and AFL (FleA) with a blood group A determinant (**B**, PDB 4AH4 [63]). Coloring is by protein chain or blade, respectively. **(C)** Close-up view of the fucose binding site 3 in AFL. **(D-F)** Surface representations of the binding of H-type 2 (D; PDB 3ZZV [60]) and Lewis^x (E, PDB 3ZW1 [60]) determinants in the intramolecular binding site of BamBL, and Lewis^y (F, PDB 4D4U [63]) in binding site 3 of AFL.



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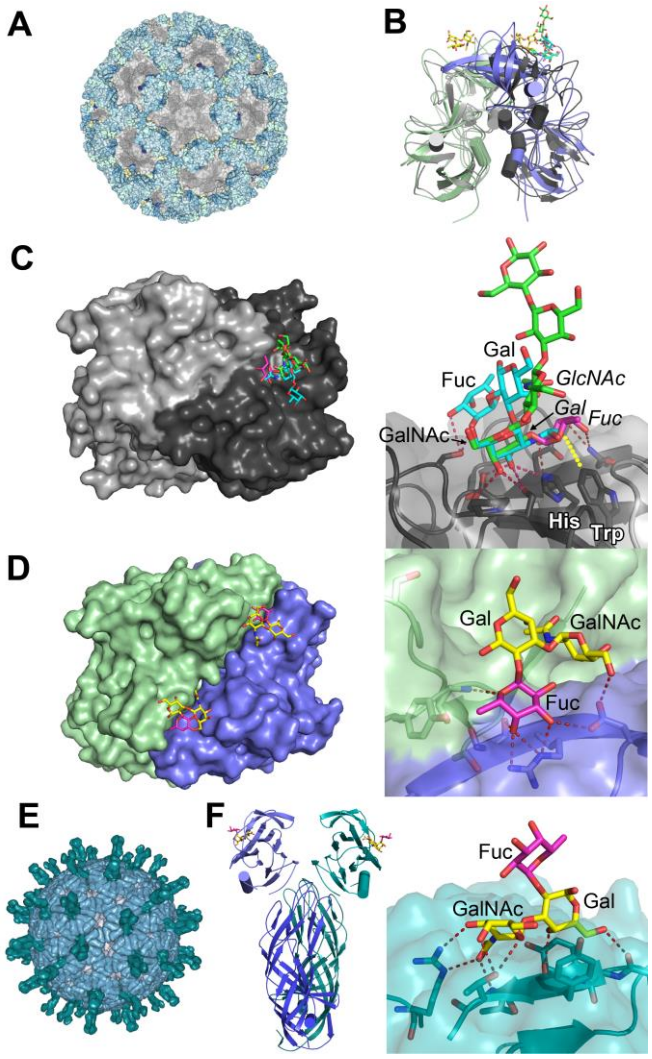

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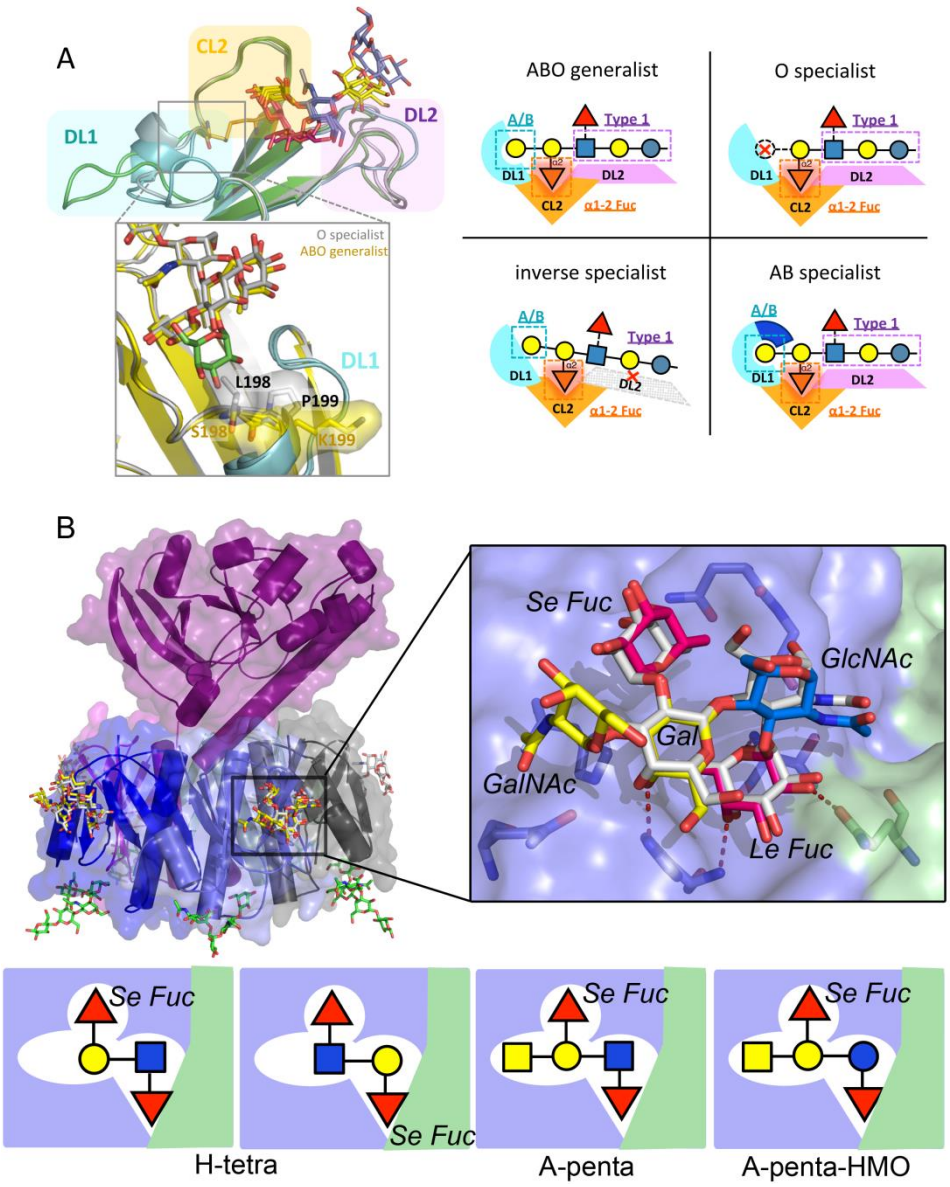

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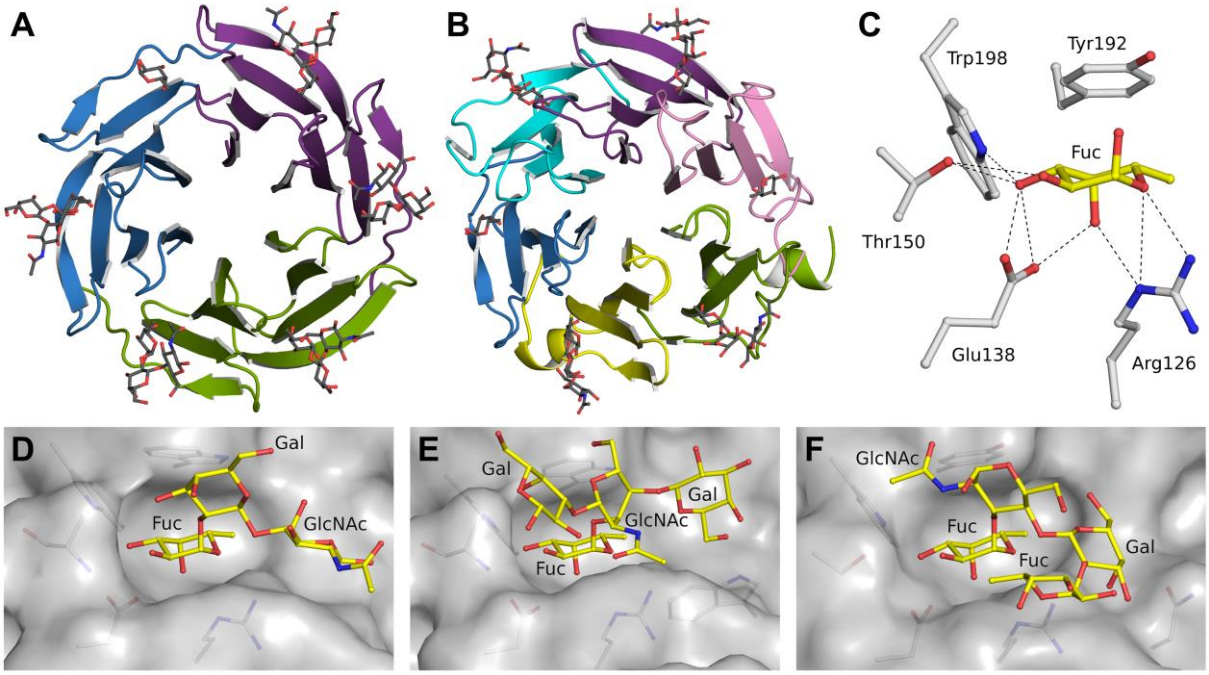

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