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Review

Mendelian resistance to human norovirus infections

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Abstract

Noroviruses have emerged as a major cause of acute gastroenteritis in humans of all ages. Despite high infectivity of the virus and lack of long-term immunity, volunteer and authentic studies has suggested the existence of inherited protective factors. Recent studies have shown that histo-blood group antigens (HBGAs) and in particular secretor status controlled by the α1,2fucosyltransferase *FUT2* gene determine susceptibility to norovirus infections, with nonsecretors (FUT2−/−), representing 20% of Europeans, being highly resistant to symptomatic infections with major strains of norovirus. Moreover, the capsid protein from distinct strains shows different HBGA specificities, suggesting a host–pathogen co-evolution driven by carbohydrate–protein interactions.

Keywords: Norovirus; FUT2; Secretor; Histo-blood group antigen; Gastroenteritis

1. Introduction

Viral infections in humans are notable for the diversity of host responses, rate of progression and disease outcomes. A large body of data indicates that these responses depend not only on viral factors but also on inherited components affecting host susceptibility. The inherited components can be part of the immune response or as discussed in this review, part of receptor polymorphism. The first step in the life cycle of virus is attachment to a specific receptor on the cell surface through which virus enters the host cell. These receptors are important determinants of virus host-range, tissue tropism and can, if the receptor gene exhibits polymorphisms, alter not only disease pattern but also susceptibility to particular pathogens.

With regard to the genetic determinisms of susceptibility or resistance to infection, two main types of mutations can be distinguished. First are rare mutations affecting the immune response to either a broad or a restricted set of pathogens. They impair various pathways of the immune response leading to either severe immunodeficiency, characterized by a high vulnerability to infection, or to selective immunodeficiency, characterized by vulnerability to one or few pathogens [1]. Given their high negative impact on the fitness of the individuals carrying them, these mutations are probably negatively selected and their presence in populations can be considered accidental. Second are polymorphisms in receptor genes exerting a strong effect on susceptibility or resistance to specific infectious agents. For example, SJL mice are significantly more resistant to a lethal dose of mouse hepatitis virus (MHV), than BALB/c mice [2]. The resistance is due to an allelic variation in the gene encoding carcinoembryonic antigen-related cell adhesion molecule 1. Another example of natural host resistance is the restriction of ecotropic murine leukemia virus (MuLV) infection by the mouse fV4 gene. In humans, a striking example is parvovirus B19 and the carbohydrate P antigen receptor, where individuals lacking the P antigen are resistant to parvo B19 infection [3]. Another human example concerns a polymorphism in the chemokine CCR5 receptor. The identification of CCR5 as a co-receptor for HIV prompted genetic screening of individuals who escape HIV disease despite risk behavior. Individuals carrying a homozygous 32 bp deletion in the coding sequence of CCR5 are extremely resistant to HIV infection because the deletion results in a frame shift and thus a nonfunctional receptor. The

Abbreviations: CCR5, chemokine receptor 5; Fuc, fucose; FUT2, α1,2fucosyltransferase; Gal, galactose; GG, genogroup; Glc, glucose; HBGA, histo-blood group antigen; HIV, human immunodeficiency virus; IgG, immunoglobulin G; Le, Lewis antigen; NAc, *N*-acetyl; NV, Norwalk virus; NTPase, nucleoside triphosphate; ORF, open reading frame; Pol, polymerase; Pro, proteinase; RHDV, Rabbit Hemorrhagic Disease Virus; RNA, ribonucleic acid; RT-PCR, reverse transcriptase polymerase chain reaction; SMV, Snow Mountain virus; VLP, virus-like particles; VP, virus protein

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CCR5Δ32 allele is found predominantly in the Caucasian population with an allele frequency of 0.1 [4]. While CCR5Δ32 provides resistance to HIV it surprisingly predisposes to West Nile virus infection [5,6].

In this review, we discuss recent novel observations indicating that HBGAs play a crucial role in determining resistance to norovirus infections, a virus that is one of the most common causes of infectious gastroenteritis [7,8].

2. Historic background to winter vomiting disease and norovirus

In 1929, Zahorsky first described "winter vomiting disease" but it took until 1972 when Kapikian first identified viruses in stools from a gastroenteritis outbreak at an elementary school in Norwalk, Ohio [9]. The virus was named Norwalk-agent and was the first virus firmly being associated with acute gastroenteritis (Fig. 1). It should be remembered that by then, there was no conclusive evidence that viruses were associated with acute gastroenteritis. Yet, acute nonbacterial gastroenteritis was repeatedly demonstrated between 1945 and 1953. Rieman et al. [10], Gordon et al. [11] and Jordan et al. [12] all inoculated volunteers with bacteria-free filtrates that resulted in infections with clinical symptoms similar to authentic Norwalk virus infections.

During the next decades, the study of norovirus was fraught with challenges. The viruses do not grow in cell or organ cultures and there is no small animal model for infection or disease and furthermore, the amount of virus that is present in stool samples is limited so that purification is problematic. Without an animal model and an *in vitro* cultivation system, our current knowledge of noroviruses is mainly derived from authentic outbreaks, volunteer studies and productions of *in vitro* assembled virus-like particles (VLPs), in mammalian and insect cells. Milestones in norovirus biology are the molecular cloning of Norwalk virus genome in the early 1990s [13] and the development of RT-PCR assays, able to take into account their diversity [14]. These assays allowed to demonstrate that the epidemiological importance of noroviruses had long been largely underestimated. Thus, a norovirus strain could be identified among 96% of nonbacterial gastroenteritis outbreaks that occurred in the USA between January 1996 and June 1997, amounting to 23 million cases [8]. This rate was as high as 80% between 2000 and 2004 [15]. Outbreaks may occur all year round but are much more frequent in wintertime, affecting people of all ages. Similar high rates were reported in other countries such as Great Britain, Sweden, Germany, Holland or Japan, indicating that noroviruses should be responsible for 60–85% of all gastroenteritis outbreaks within developed countries [16–18].

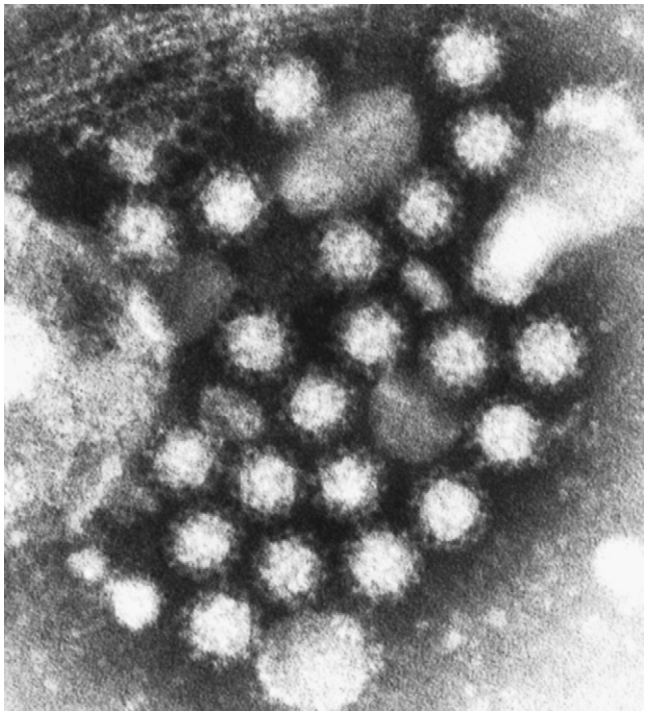

Fig. 1. Electron microscopy of noroviruses (29 nm) from clinical sample. Virus particles visualized by negative staining (phosphotungstic acid).

3. Noroviruses structure, classification and diversity

Noroviruses are nonenveloped and their polyadenylated positive strand RNA genome is 7.7 kb long [19]. The genome is organized in three open reading frames (ORF). Located in 5′, ORF1 encodes nonstructural proteins: NTPase, Vpg, pro and pol (Fig. 2), ORF2 encodes the unique capsid protein VP1 of about 60 kDa, and ORF3 in the 3′ end encodes a small protein of 20 kDa called VP2 that probably plays a role in the expression and stability of VP1 (Fig. 2).

Sequence comparisons of the RNA-dependent RNA polymerase and of the capsid protein from many patients and animals allows classification of noroviruses into 5 genetic groups and at least 29 genetic clusters: 8 in genogroup I (GGI), 17 in GGII, 2 in GGIII, and 1 in GGIV and GGV (Fig. 3). Human strains are classified into genogroups I, II and IV, with porcine and bovine strains classified into genogroups II and III, respectively and the recently described mouse strain is so far the only member of genogroup V [20].

In the VP1 capsid protein, human noroviruses strains share at least 60% amino acid sequence identity within the same genogroup, whereas most if not all GGI strains share less than

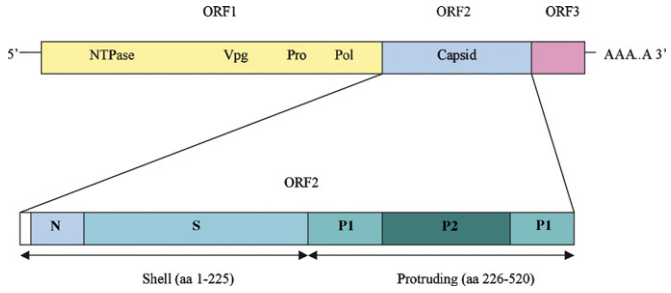

Fig. 2. Schematic representation of the noroviral genome, which is divided into three open reading frames: ORF1–3. ORF1 encodes four nonstructural proteins, namely NTPase (nucleoside triphosphate) VPg, proteinase and polymerase. ORF2 encodes the capsid of the virus and ORF3 encodes a minor structural protein. Bottom part: Schematic illustration of ORF2, encoding the capsid of the norovirus. N is the NH_2-terminal arm of ORF2 and S refers to the inner shell; the most conserved part of the capsid. P1 and P2 are the protruding parts, of which P2 is the most variable and exposed.

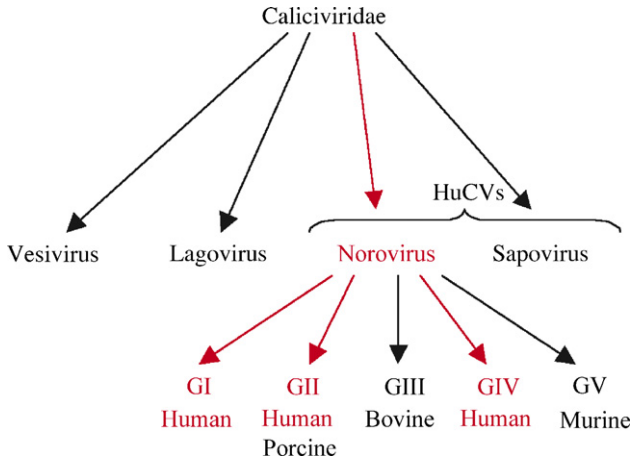

Fig. 3. The caliciviruses are divided into four different genera: Lagovirus, Vesivirus, Sapovirus and Norovirus of which the last two can infect humans. Noroviruses is further subdivided into five distinct genogroups, GGI–GGV.

50% amino acid identity with those in GGII (Fig. 4) [21]. Noroviruses can be further subdivided into clusters. Norovirus strains within a genetic cluster share at least 80% VP1 amino acid sequence homology with the cluster reference strain. While sequencing have allowed simple classification of noroviruses, a more complete biological understanding of different strains will require that biological and functional properties are considered as well.

The structure of the Norwalk strain capsid has been solved by X-ray crystallography and is arranged into $T = 3$ icosahedral symmetry. The virion is composed of 90 dimers of the major VP1 protein. The VP1 capsid protein can be divided into three domains, the N-terminal shell domain, buried inside the capsid, the intermediate S-domain and the protruding P. The N-terminal 225 amino acids constitute the S-domain of VP1 and the motifs essential for formation of the icosahedral capsid shell. The P-domain is composed of the remaining amino acids divided into two major sub-domains: P1 and P2, with the P1 sub-domain being implicated in the antigenicity of norovirus [22,23], while the P2 sub-domain has been suggested to bind to the cellular receptor (Fig. 5) [24].

The VP2 protein exhibits extensive sequence variability. While the function for several noroviruses proteins have been established, the role of VP2 in the replication process is yet unsolved, although it is clear that VP2 is a minor structural protein present in one or two copies per virion. A role of VP2 in RNA genome packing is proposed based on the fact that VP2 is a basic protein. However this is not supported by experimental data.

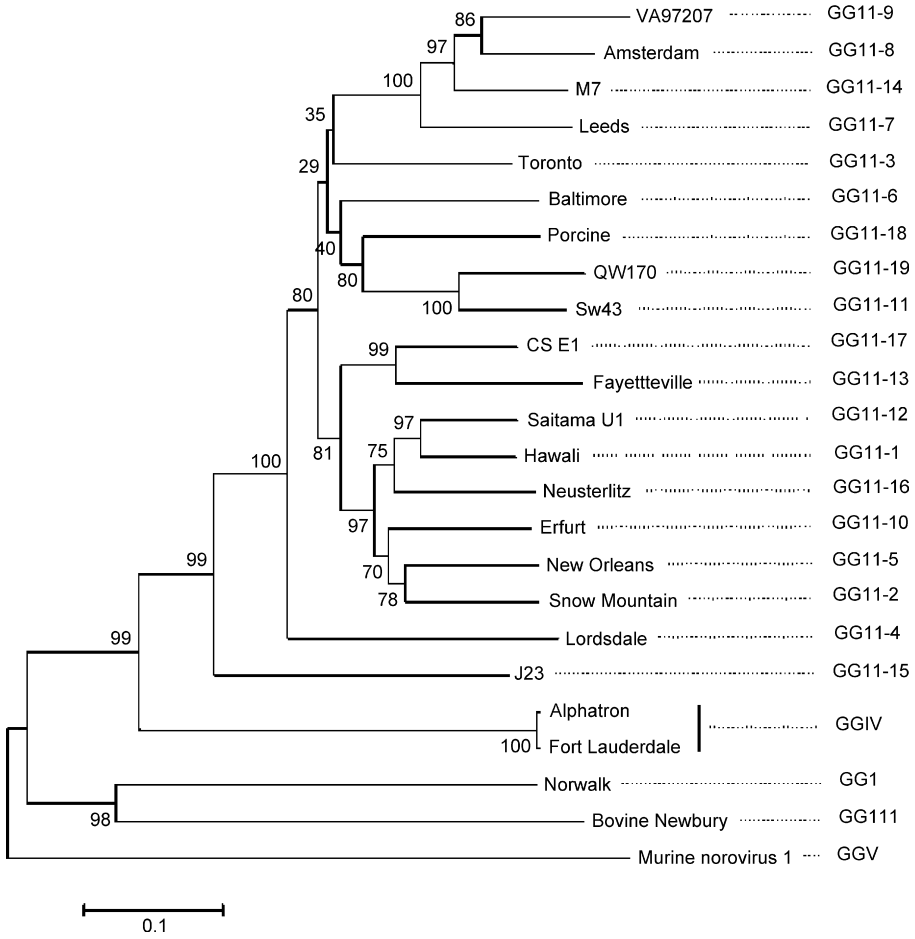

Fig. 4. Neighbor-joining phylogenetic tree of norovirus genogroups based on the complete capsid region. Genogroups (GG) and genotypes (numbers after GG) are indicated. Percentage bootstrap values are given at branch nodes, and the number of substitutions per site is indicated by the scale bar.

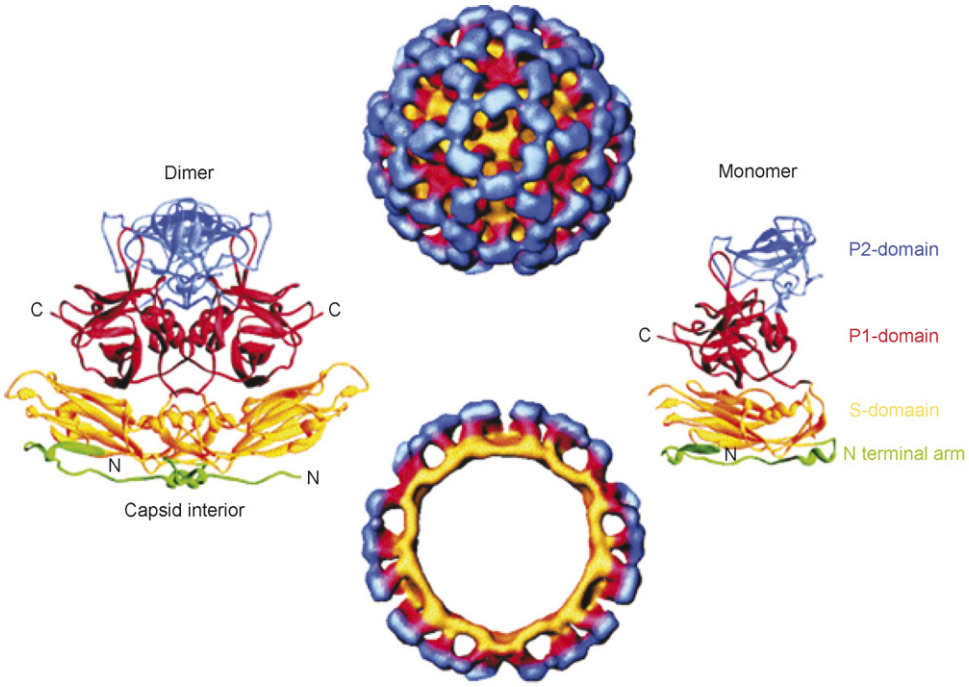

Fig. 5. Cryo-electron microscopy (22 Å) and X-ray crystallography (3.4 Å) of Norwalk virus-like particles. Surface representation (top) and cross-section. The virus-like particles have 90 dimers of the capsid (left ribbon diagram) in a $T = 3$ icosahedral symmetry. The capsid protein is divided into an N-terminal region (green) facing the interior of the VLP, a shell domain (S-domain, yellow) that constitutes the surface of the virus, and a protruding domain (P-domain) that emanates from the S-domain surface. The P-domain is divided into P1 and P2 (red and blue, respectively) with the P2-domain at the most distal surface of the particle. With permission from Refs. [74,102,103].

4. Transmission and clinical symptoms

Noroviruses are highly aggressive as illustrated by high antibody prevalence, high attack rates [25], very low infectious dose and documented asymptomatic shedding. These viruses are transmitted by the person-to-person route, after consumption of contaminated food [25] (fresh fruit, vegetables, raw oysters and bakery products), water (drinking, ice or swimming) and following exposure to contaminated environmental surfaces and to airborne vomitus droplets [25–33].

The disease is characterized by nausea, vomiting, diarrhea, abdominal cramps, and by low-grade fever, symptoms that appear 12–48 h post-infection and usually regress within 2–3 days. While the disease usually is short lasted, complications have been observed in infants and immuno-compromised individuals; the latter can shed virus for years [34] and an infant who received an intestinal transplant developed persistent norovirus-associated diarrhea, which could be resolved only after reduction of immunosuppressive therapy [35]. Furthermore, an unusual outbreak occurred among British soldiers engaged in Afghanistan with four infected soldiers displaying uncommon and severe symptoms such as neck stiffness, light sensitivity and confusion that required emergency assistance [36]. Besides heavy dehydration, these even included one case of intravascular disseminated coagulation, suggesting that for individuals in stressful situations, noroviruses may cause fatal diseases in absence of intensive care. Information about the pathogenesis is limited but volunteer studies have shown that Norwalk virus infects the proximal small intestine that is accompanied by transient (<2 weeks) D-xylose malabsorption [37,38].

5. Are noroviruses emerging?

Recent reports indicate that novel norovirus strains can emerge either nationally or globally and increase disease incidence [39,40]. In 2002, a new virus variant spread in Europe, affecting a large number of countries [41]. Furthermore a new virus variant was also associated with an outbreak on a cruise in the United States [42]. Why these strains emerge is unclear but it is interesting to note that they all belong to the genogroup II, cluster 4, that predominates in the world [39,43,44]. Currently it is not known if the dominating GGII/4 strain cause more severe symptoms than other strains or belong to a new and emerging serotype of norovirus with unique biological properties [45].

6. Is a host genetic factor associated with resistance to norovirus infections?

Sero-epidemiological studies have shown antibody prevalences to norovirus of >80% in various studies. While the antibody prevalence level is similar to that of rotavirus, another common intestinal pathogen, the epidemiology is different. In contrast to rotavirus where the symptomatic period is before school age, all age groups are susceptible to norovirus infections, suggesting lack of protective immunity and/or a large number of antigenetically distinct viruses. Several groups [46,47] have found that serum antibody titers >1:100 were associated with protection of children against Norwalk virus infection. However, this pattern was not found in volunteer studies among adults. Blacklow et al. [48] reported that ill

volunteers challenged with Norwalk virus were more likely to have high than low serum antibody titers, and Parrino et al. [49] showed that serum antibody titers to Norwalk virus were not protective against illness. They found that 6/12 individuals challenged with Norwalk virus developed clinical symptoms, whereas the other 6 persons remained asymptomatic. When the same 12 individuals were re-challenged with the same inoculum, 1–2 years later the same volunteers who were ill at the first time experienced clinical symptoms again, whereas those who remained asymptomatic after the first challenge remained resistant again. Additional studies have confirmed the observation of individuals being resistant to norovirus infection [50,51]. Furthermore, an early observation from an authentic outbreak revealed that individuals with resistance clustered in families, even with the same exposure of pathogen [52].

Moreover, Johnson et al. [53] found that pre-existing serum antibodies to norovirus do not seem to be associated with protective immunity. Indeed, those with high initial serum antibody titers were significantly more likely to become ill. While support of long lasting immunity could not be documented, short-term resistance have been observed [49,53,54].

7. The Norwalk virus (NV) carbohydrate ligands

Carbohydrates of membrane glycoproteins or glycolipids are often used by viruses to attach to cell surfaces (Table 1). Numerous viruses use the negative charges of sialic acids or of heparan sulfates for binding but few examples of neutral sugars used as ligands by viruses have been described so far. Taking profit of the ability of a rabbit calicivirus (RHDV) to agglutinate human erythrocytes, we could demonstrate some years ago that this virus could bind specifically to a neutral trisaccharide of the histo-blood group family [55]. The trisaccharide, Fucα2Galβ4GlcNAc, called the H type 2 antigen, is present in large amounts on human erythrocytes and on rabbit epithelial cells. It was shown by immunohistochemical methods that RHDV binds to rabbit epithelial cells of the upper airways and of the gut through this trisaccharide. Although it has not yet been demonstrated if this attachment is required for infection, the observation led to the search of similar ligands for the human prototype calicivirus, the GGI Norwalk virus strain.

Table 1
Viruses that use carbohydrates as receptors

Virus	Carbohydrate
Adeno-associated virus	Heparan sulfate
Coronavirus	Sialic acid
Flavivirus	Heparan sulfate
Herpes viruses	Heparan sulfate
Noroviruses	ABH, Lewis antigens
Orthomyxo viruses	Sialic acid
Paramyxo viruses	Sialic acid
Parvovirus B19	P antigen
Polyomavirus	Sialic acid
Rabbit calicivirus	ABH
Reovirus	Sialic acid
Rotavirus	Sialic acid

We observed that another trisaccharide Fucα2Galβ3GlcNAc, very similar to H type 2 and called H type 1, was recognized by norovirus VLPs [56]. In addition, closely related structures called H type 3 and Le^b proved to be recognized by NV VLP as well [56–58]. These oligosaccharides have an α1,2-linked fucose in common and they are present on gut surface on epithelial cells. *In vitro* experiments showed that they are used by VLPs for attachment to the cell surface and that the binding is followed by internalization of the artificial capsids [56]. It indicated that H type 1, Le^b and to a lesser extent H type 3 were ligands for NV capsids but it was not yet possible to conclude that the interaction between these ligands and the virus represented a first step of the infection process. Despite the absence of a cell culture model, this aspect could nevertheless be studied thanks to the genetic polymorphism of the expression of the α1,2-linked fucose on gut surface epithelial cells.

8. Mendelian resistance to Norwalk virus infection

The H types 1, 2 or 3 and Le^b carbohydrate structures are histo-blood group antigens (HBGAs). These antigens are synthesized by the sequential addition of monosaccharides to precursor oligosaccharides that constitute the peripheral region of glycolipids as well as of O- and N-linked glycans of glycoproteins as depicted in Fig. 6. The expression of α1,2-linked fucose residue on surface epithelial cells of the gut and in body fluids (hence the term "secretor") is dependant upon the presence of a wild type *FUT2* allele. The *FUT2* gene, also called the *Secretor* gene, encodes an α1,2fucosyltransferase and in the homozygous state, null mutant alleles lead to an absence of the α1,2-linked fucose residue, characterizing the so-called nonsecretor phenotype [59]. Various null *FUT2* alleles have been described that widely differ in frequency among human populations [60,61]. In the European population, the most frequent null allele (se^{428}) is characterized by the 428G > A nonsense mutation. This mutation is found in over 95% of European null alleles. It is also found in the most frequent null alleles among Africans but is not present in Asia [62]. Homozygous nonsecretors (*FUT2*−/−) represent as much as 20% of the European population [4,45].

By immunohistochemistry we showed that NV VLP attach to gut surface epithelial cells of Secretor-positive individuals, whereas they do not bind to the tissues of nonsecretors (FUT2−/−) [56]. It was thus most interesting to investigate if nonsecretors, devoid of the ligand for recombinant capsids, would be resistant to infection by NV. This was done in two studies by determining the secretor status and the *FUT2* genotype in volunteers challenged with the Norwalk strain [63,64]. From both studies it was concluded that nonsecretors (*FUT2*−/−) were not infected by Norwalk virus since none of the 30 volunteers of this group was ill, showed an increase in anti-NV circulating antibodies or had detectable virus RNA in feces. In contrast, 76 out of 97 Secretors (*FUT2*+/− or *FUT2*+/+) developed a strong anti-NV antibody response, and excreted virus (viral RNA). These results unequivocally demonstrated that the presence of the *FUT2*-dependant α1,2-linked fucose was required for NV infection and that a significant fraction of the population was genetically resistant.

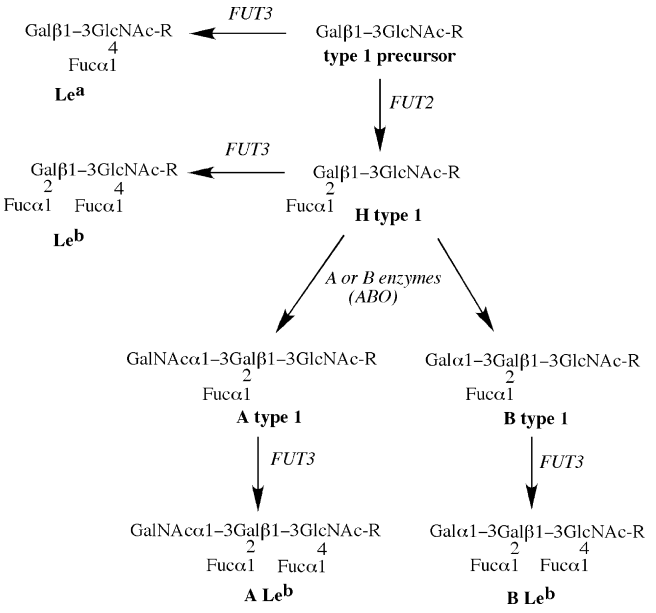

Fig. 6. Biosynthesis of ABH and Lewis histo-blood group antigens (HBGAs) proceeds by stepwise addition of monosaccharides to precursor structures. The figure shows the example of the so-called type 1 precursor Galβ1-3GlcNAc. H antigen synthesis is under dependence of the *FUT2* gene encoding an α1,2fucosyltransferase which attaches a fucose residue in α1,2 linkage to the terminal galactose of the precursor. Inactivating mutations of *FUT2* are responsible for a phenotype called "nonsecretor", characterized by the lack of α1,2-linked fucose-containing HBGAs on many epithelial cells and in secretions such as saliva. Synthesis of the A and B antigens requires the previous synthesis of the H antigen which will be used as an acceptor substrate by the A and B enzymes encoded by the *A* and *B* alleles at the *ABO* locus. These enzymes will add an *N*-acetylgalactosamine or a galactose in α1,3 linkage on the galactose residue of the H antigen to give the A and B antigens, respectively. *O* alleles at the *ABO* locus correspond to inactivated alleles that cannot encode for a functional glycosyltransferase. The Lewis antigens are synthesized thanks to α3/4fucosyltransferases that attach a fucose residue on the *N*-acetylglucosamine of the precursor. In the case of type 1 precursor, this fucose residue is in α1,4 linkage and its addition gives the Lewis a (Le^a) and Lewis b (Le^b) antigens. The gene mainly responsible for their synthesis in epithelia is *FUT3* for which, similar to *FUT2* and *ABO*, inactivated alleles are known. Individual homozygous for inactivated *FUT3* alleles (*FUT3*−/−) are essentially devoid of α4-linked fucose on their epithelial cells and in saliva and their phenotype is usually referred to as "Lewis negative". Enzymes are in italics and the antigens are named in bold. Gal: galactose; GlcNAc: *N*-acetylglucosamine; Fuc: fucose; GalNAc: *N*-acetylgalactosamine.

As shown in Fig. 6, the H type 1 structure that corresponds to the minimal ligand recognized by NV can be modified by further addition of monosaccharides. A second fucose residue can be added by the FUT3 enzyme, also called the Lewis enzyme, generating the Le^b antigen. Likewise, either an *N*-acetylgalactosamine or a galactose can be added by the A or B enzymes, respectively encoded by the *A* and *B* alleles of the *ABO* gene. Interestingly, it was observed in both volunteers studies cited above that FUT2 positive individuals of the B blood group type were less likely to be infected than A or O individuals or when infected, they were more likely to remain asymptomatic [63,64]. This could possibly be explained by a masking of the H type 1 receptor by the presence of the galactose residue added by the B enzyme. In agreement with this hypothesis we observed weak binding of NV VLPs to salivary mucins from Secretor B blood group individuals compared to A or O individuals, as well as decreased binding to FUT2 and B allele co-transfected CHO cells compared to FUT2-only transfected cells [65].

Thus, alleles at the *FUT2* locus determine sensitivity or resistance to the Norwalk virus strain and the polymorphism at the *ABO* locus modulates sensitivity within the Secretor-positive group.

9. The nonsecretor phenotype provides resistance to authentic norovirus infections by GGII strains

Since evidence for the role of the *FUT2* gene polymorphism in norovirus infection was first obtained with the less common genogroup I Norwalk virus that is rarely found in authentic outbreaks, it was important to investigate if the same phenomenon would be valid also in outbreaks associated with global dominating genotypes II norovirus strains [39,43,45]. To address this question, the role of the *FUT2* polymorphism and secretor status was investigated in norovirus outbreaks in Sweden. Allelic polymorphism characterization at nucleotide 428 from symptomatic ($n = 53$) and asymptomatic ($n = 62$) individuals associated with nosocomial and sporadic norovirus outbreaks revealed that homozygous nonsense mutation (428G > A) in *FUT2* segregated with complete resistance to the disease. Of all symptomatic individuals, 49% were homozygous (*FUT2*+/+) and 51% heterozygous secretors (*FUT2*+/−) and none were secretor-negative (*FUT2*−/−), in contrast to 20% non-secretors (*FUT2*−/−) among Swedish blood donors ($n = 104$) ($p < 0.0002$) and 29% in asymptomatic individuals associated with nosocomial outbreaks ($p < 0.00001$). In addition, it was shown that the virus strains from these outbreaks could bind to saliva mucins from FUT2 positive individuals who express the α1,2-linked fucose but not from *FUT2*−/− individuals who lack this carbohydrate, clearly linking their genetic resistance to the lack of ligand for the virus [45].

Since both the volunteer studies and the authentic outbreak studies revealed a strong correlation between secretor status and VLPs binding to salivary mucins carbohydrates, it is reasonable to assume that carbohydrate-VLPs binding analysis will provide information on norovirus-HBGA interactions valid for authentic infections.

As a result, a relatively large set of VLPs from distinct genogroup I and II strains have now been analyzed by several teams. The binding to carbohydrates has been studied using various methods such as a saliva mucin assay where the attachment of capsids to saliva from individuals of different FUT2, as well as ABO and FUT3 status is detected [57,66,67]. Various assays developed to detect VLPs binding to synthetic oligosaccharides have also been used [67,68] and finally it was possible to test the ability of some VLPs to recognize ABH antigens by hemagglutination since VLPs from some noroviruses strains can agglutinate human red cells at 4 °C [58]. To our knowledge, VLPs from 17 strains have been tested in at least one of these assays. All of them demonstrated some binding to one HBGA structure or more but they showed very distinct specificities. Since the presence of distinct carbohydrates structures is dependent upon the combined polymorphism at the *FUT2*, *FUT3* and *ABO* loci as described

in Fig. 6, it is possible to deduce the population subgroup that a strain is expected to target from its carbohydrate-binding specificity. The results are shown in Fig. 7A and B. Most virus strains recognize carbohydrates present on gut epithelial cells from Secretor (*FUT2*+/+ or *FUT2*+/−) individuals, in accordance with the studies reported above. However, among these strains, the influence of the ABO phenotype was more or less extended. Some strains could bind to saliva from Secretor individuals irrespective of the ABO phenotype, some others like NV bound to saliva from O and A Secretor individuals but not or less so from B individuals as described above. Yet, some other strains bound to saliva from Secretor individuals of either A or B blood groups but not, or much less, if they were of the O blood group. This could be confirmed by hemagglutination using red cells of different ABO groups. There are strains that did not bind to the natural saliva mucin oligosaccharides but that could attach to synthetic oligosaccharides corresponding to HBGA structures, allowing to classify them in terms of expected pattern of infectivity [67]. Overall the results indicated that not a single strain could cover the whole human HBGAs diversity, although collectively noroviruses almost succeeded to cover the diversity. The only notable exception concerns the subgroup of individuals who are both FUT2−/− and FUT3−/−. Individuals with this genotype do not add a fucose residue to the carbohydrate precursor structure of their epithelial cells (Fig. 6) and so far, none of the strains studied was able to bind in absence of at least one fucose residue. Individuals with this genotype are rare [60,69]. In Europe, they represent about 2% of the population and there is no population described with a much higher frequency of this genotype. Thus noroviruses are collectively expected to be able to infect nearly everybody, in accordance with the presence of anti-norovirus IgG in the serum of 95–100% adults. Yet, only a fraction of the population is expected to be targeted by a given strain.

As previously reviewed by others [24], the ability of norovirus strains to bind to HBGA structures does not clearly match with the genetic classification into genogroups and clusters. However, it is interesting to note that strains of the genogroup II, cluster 4 that largely dominate in recent outbreaks both in Europe and the USA cover the largest HBGAs spectrum. Thus, the three strains of this type tested so far using VLPs are the Grimsby, the VA387 and the Dijon95 strain (unpublished results) and all bind to saliva mucins from secretor-positive individuals irrespective of the ABO character. This is in accordance with the observations from the Swedish outbreaks where the norovirus strains belonged to the same cluster and where only Secretor individuals where infected [45].

The impact of the ABO phenotype on norovirus infection has been reported without mention of the FUT2 status from different outbreaks yielding contradictory results, which can now be explained. One study on British soldiers engaged in Afghanistan showed an overrepresentation of O blood group individuals, whereas A individuals and even more so, B individuals were underrepresented among the infected [36]. Inversely, an outbreak occurring in a German hospital affected mainly A individuals [70]. Likewise, another recent study from Japan reported that blood type A children had been more susceptible

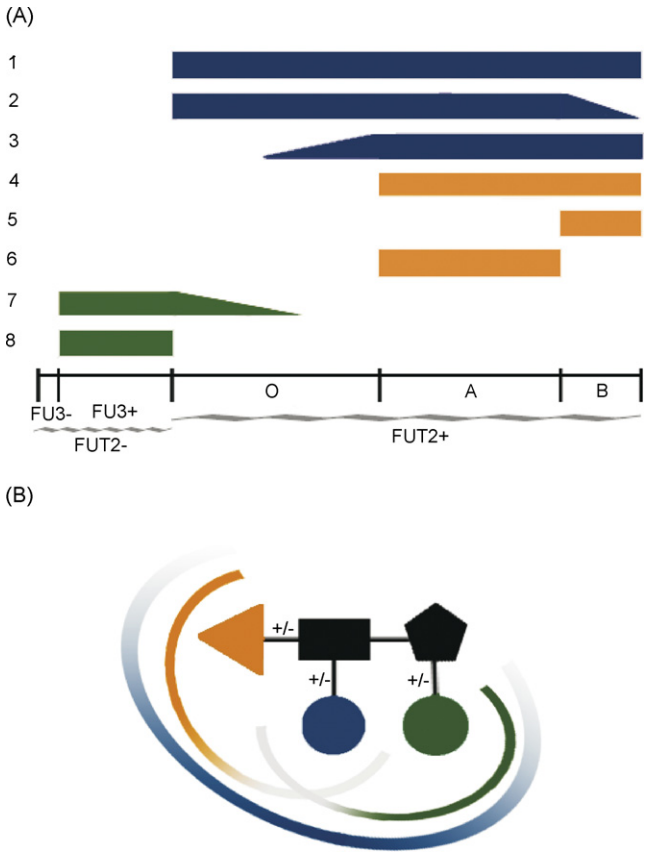

Fig. 7. Human infectivity spectrum deduced from the carbohydrate-binding specificities of noroviruses strains. (A) The human population is divided into subgroups depending upon the combined polymorphisms at the *FUT2*, *ABO* and *FUT3* loci. For simplicity, the Lewis negative phenotype (*FUT3*−/−) is not shown within the Secretor (FUT2+) subgroup. Phenotypic subdivisions are shown in the figure according to approximate frequencies observed in Western Europe. Significant differences in allelic frequencies at each locus can be observed in different parts of the world. Eight types of norovirus strains are depicted according to their ability to bind distinct HBGAs structures. Type 1 corresponds to the VA387 and Grimsby strains, type 2 to NV, type 3 to Mexico, type 4 to MOH, type 5 to SMV, type 6 to BUDS, type 7 to VA207 and Boxer, type 8 to OIF. Since the presence of a given carbohydrate depends upon the genetic polymorphism, the spectrum of individuals that one type of strain can recognize is limited to a fraction of the population. Cross-reactivities may occur as exemplified by strains of the types 2, 3 and 7 that can extend their host recognition beyond their main target phenotype. In the case of type 2 strains exemplified by the Norwalk strain, blood group B individuals are less well recognized. They are less likely infected and if so, they can remain asymptomatic. In the case of type 7 strains, although the main targets should be nonsecretors/Lewis positive individuals (FUT2−/FUT3+), some blood group O Secretors (FUT2+) should be infected providing they are Lewis positive (FUT3+). (B) Monosaccharides units at three positions can be subject to a polymorphism and the eight types of strains depicted in A can be explained by the preferential targeting of the monosaccharide residue at one of these three variable positions. The fucose residues (circles) are present or absent depending upon the *FUT2* and *FUT3* genes polymorphisms (blue and green respectively). The *N*-acetylgalactosamine or galactose (brown triangle) can be either present or absent depending upon the *ABO* gene polymorphism. Some strains will target mainly the α1,2-linked fucose (types 1 and 2), others will target mainly the *N*-acetylgalactosamine or the galactose residue (types 3–6), while others yet will target mainly the α1,4-linked fucose (types 7 and 8). These major specificities are figured by dark colors of arc of circles. Strain binding specificity can extend more or less beyond the main target epitope (arc of circles lighter colors). The figures correspond to interpretation of data obtained by different methods and reported in Refs. [58,65–68].

to a norovirus outbreak that occurred in a junior high school [71] whereas a study from Switzerland did not find any relationship between infection and the ABO type [72]. These seemingly contradictory observations can now be understood in the light of the strain HBGA-binding pattern diversity as depicted in Fig. 7A. If distinct strains with distinct HBGA-binding patterns were responsible for these outbreaks, it is not surprising that different subgroups of the population were the main targets in each outbreak. At present, there is one discrepant study on volunteers that should be mentioned since it challenges the concept that all human noroviruses use HBGAs to infect their host. The SMV strain was given to a group of volunteers and no association between the FUT2 or ABO status was observed [63], although SMV VLPs bind to synthetic HBGAs with a pattern suggesting that the strain should infect preferentially individuals of the B blood group within the FUT2 subgroup [67]. The lack of association between infection and HBGA type suggests that SMV may use another primary ligand to infect. It would then be most reasonable to believe that this strain should be common in the society, as virtually everyone would be susceptible. Yet, not only is it seldom identified in authentic outbreaks [73] but it shows a limited clinical attack rate in volunteers [63]. It should be noted that the study included a limited number of volunteers, 17 in total, so that their small number in each subgroup could hide an effect that would not be a strict all or nothing phenomenon as suggested by the binding pattern of some strains that show cross-reactivities with HBGA shared among distinct human *ABO*, *FUT2* and *FUT3* genotypes. To test this interpretation, future studies are warranted where the HBGA-binding pattern of the strain responsible for the outbreak should be examined together with the combined genotype and/or phenotype as was done in the study of Swedish hospital outbreaks [45].

10. The carbohydrate-binding site on the capsid

The P2 sub-domain of the capsid protein is protruding from the core of the capsid with an arch-like shape [74]. Sequence comparison of capsids from various strains reveals that the P2 region concentrates a lot of the strain variability [20]. Given its localization at the capsid surface, it is thus reasonable to believe that this sub-domain should be under immune selective pressure, possibly resulting in the generation of escape mutants. The epitope recognized by a monoclonal antibody that could inhibit attachment of NV capsids to differentiated Caco-2 cells was mapped to the P2 sub-domain [75]. The binding of these capsids to differentiated Caco-2 cells was later shown to involve recognition of the H type 1 cell surface antigen, suggesting that the carbohydrate-binding site was located in this region of the capsid protein [56]. Furthermore accumulation of mutations in the P2 region has been observed in chronic virus shedding individuals [34]. Recombinant P-domains can form dimers or 24 mers depending upon whether the hinge region that links the P-domain to the S-domain is maintained. Both the dimers and the 24 mers particles maintain the strain carbohydrate specificity, unambiguously showing that the carbohydrate-binding site is located on the P-domain [76,77]. Modeling revealed a plausible binding pocket formed by an RGD conserved motif and some surrounding strain-specific amino acids. Site-directed mutagenesis analysis revealed the importance of this binding pocket for HBGAs recognition for distinct strains [78,79]. A second plausible binding site located at 10 Å from the first one has also been uncovered and validated by site-directed mutagenesis [79,80]. In addition, it was recently reported that a blocking monoclonal antibody recognizes a linear epitope of the NV capsid that corresponds to amino acids located near the first potential binding site mentioned above. Thus, although it is not yet clear at this stage whether there is a single carbohydrate-binding site or more closely located binding sites that could act in concert for recognition and attachment to HBGAs, as suggested by Tan and Jiang [24], the binding interface between noroviruses and their carbohydrate ligands has now been mapped to a restricted surface of the P2 sub-domain.

Mutations in the P2 sub-domain that allow escape from neutralizing antibodies, may not necessary prevent recognition of virus to cell surface as more than one receptor might be required for infection. Yet, the immune selective pressure could drive variability in terms of carbohydrate-binding specificity. This could explain the lack of direct relationship between genotypic strain classification and specific HBGAs recognition.

11. The role of secretor status in infectious diseases

Apart from norovirus, secretor status is also known to affect susceptibility towards other infectious agents (Table 2). Secretor-negative individuals seem to be predisposed to infections by *Haemophilus influenzae*, *Neisseria meningitidis* and *Streptococcus pneumoniae* [81,82] as well as urinary tract infection caused by *Escherichia coli* [83–86]. The latter is explained by the adhesion of uropathogenic *E. coli* strains to galactosyl-globoside or its sialylated derivative. These glycolipids are found in large amount in the genitourinary tract of nonsecretor (*FUT2−/−*) women. When the FUT2 enzyme is present, these glycolipids are fucosylated, hindering recognition by the bacterial adhesin [84]. In contrast to the bacterial infections, secretors are significantly overrepresented among patients from whom influenza viruses A and B, rhinoviruses, respiratory

Table 2
Association between secretor status and susceptibility to infectious diseases

Infection	Susceptibility		Reference
	Secretor	Nonsecretor	
Haemophilus influenzae		+	[81]
Neisseria meningitis		+	[82]
Streptococcus pneumoniae		+	[82]
Urinary tract infection with *Escherichia coli*		+	[83,84]
Influenza A and B	+		[87]
Rhinovirus	+		[87]
Respiratory syncytical virus (RS)	+		[87]
Echovirus	+		[87]
HIV infection (heterosexual transmission)	+		[88,89]
HIV (disease progression)	+		[4]

syncytial virus and echoviruses had been isolated compared with the distribution of secretors in the local population [87]. Likewise, secretor-negative individuals seem to be less susceptible to HIV infection. A study of Senegalese commercial sex workers found that nonsecretors were at lower risk of getting infected by HIV-1 [88], which supports a previous study of a heterosexual HIV transmission [89]. More recently we have shown that there is an association between the secretor-negative genotype and a slower than expected disease progression among HIV-1 infected individuals [4]. This indicates that the same common polymorphisms can be involved in the determinism of Mendelian inheritance of sensitivity to several unrelated pathogens.

12. A scenario of host–pathogen co-evolution

The observation that microbes frequently use carbohydrates as receptors (Table 1) and that genes encoding tissue antigens exhibit polymorphism with significant ethnic and geographical association may have led to a selection process of microbes adapted to a certain geographical region or ethnic group, such as described for the BabA adhesion molecule of *Helicobacter pylori*. Aspholm-Hurtig et al. [90] found that the carbohydrate-binding characteristics of *H. pylori* isolates correlate with distributions of ABO phenotypes in various populations, suggesting adaptation of the pathogen to the host polymorphism.

A correlate of co-evolution between a host and a pathogen is that susceptibility to the pathogen of a given host genotype should be linked to specific variations of the pathogen. Thus an allele conferring protection to one strain (genotype) of the pathogen may confer susceptibility to another strain. In other words, as stated by Woolhouse et al., a "good" gene in one time and place may be a "bad" gene in another time and place [91]. This typically corresponds to the situation encountered for noroviruses since a large fraction of the population can be considered as immunodeficient for most strains of noroviruses while they are expected to be resistant to other strains. While nonsecretors (*FUT2*−/−) and B blood group individuals are not sensitive or are partly resistant to the NV strain respectively, they are expected to be susceptible to strains that recognize the B blood group antigen or target more specifically the Lewis antigens under control of the *FUT3* gene. The set of common alleles at the three loci *ABO*, *FUT2* and *FUT3* result in the generation of various carbohydrate ligands that fractionate the human population. The first consequence of this diversity is that part of the population is naturally resistant to strains of the pathogen that use this family of carbohydrates as primary receptor. This is similar to the protection mechanism acting at the population level that has been proposed for other common polymorphisms such as hemoglobin or the Duffy blood group variants [92]. Yet, there could be a second consequence to the carbohydrate diversity generated by the combined *ABO*, *FUT2* and *FUT3* polymorphisms in relation to rapidly evolving pathogens such as noroviruses. Let us suppose the occurrence, long ago, of an emerging highly virulent strain of a virus causing an acute disease in a host population. The virulence is such that the host population can be decimated in a short period of time, when virulence is defined as the host mortality induced by the pathogen. In this new situation, the transmission of the virus will be much less efficient and mutant strains of lower virulence will have a selective advantage and will rapidly dominate. This is in accordance with the classical "trade-off" model of virulence evolution [93], which although debated in its generality [94], appears coherent with the calicivirus–host interaction where a high replication rate is associated with a high transmissibility and may lead to a high mortality of the infected host. Natural selection will select strains with the optimal compromise between transmissibility and virulence. This new context of suboptimal virulence of the pathogen will allow the host population to recover. If some individuals were genetically resistant, their frequency will have greatly increased after recovery of the population. When the resistance allele corresponds to a mutated glycosyltransferase gene such as an *ABO* allele, the resistant individuals are not devoid of potential binding sites altogether. Providing they are Secretors, they have α1,2fucosylated structures on their epithelial cells. If they were nonsecretors, they still could have α1,4fucosylated structures as long as they are not *FUT3*−/−. This will give opportunities for mutant strains of low or moderate virulence to recognize and infect them. This co-evolution scenario will lead to a situation where subgroups of the population are resistant to some strains but sensitive to others and where not all subgroups are either sensitive or resistant to the same strains. The population is fractionated for any given strain of the pathogen. This has a cost in terms of transmission of the pathogen since many host inter-individual contacts will be unproductive for the virus. Highly virulent strains will be less likely to replace strains of moderate or low virulence [91]. The outcome of the common polymorphisms at the *ABO*, *FUT2* and *FUT3* loci would thus not only be to protect parts of the population from any given strain, but also to stabilize virulence at a moderate to low level. Such a system would contribute to protect the population even though it may seem poorly efficient at the individual level. Although this scenario is only speculative at the moment, it is worth noting that noroviruses are pathogens of low virulence. Their very high diversity is strongly suggestive of a long co-evolution with the human species. In addition, they represent a unique case of pathogen where each strain can only infect a host genotype defined by common polymorphisms of three glycosyltransferases genes with epistatic interactions. It is also noteworthy that analyses of the *FUT2* and *ABO* gene polymorphisms indicate that natural selection and not only genetic drift has been responsible for the generation of their contemporary high diversity in human populations [62,95]. Noroviruses belong to the Caliciviridae family and there is at least one emergent virus member of this family that has proved extremely virulent, namely the RHDV, which first appeared in 1984 and decimates wild rabbits of the *Oryctolagus cuniculus* species. Twelve to 48 h after infection, animals die from disseminated intravascular coagulation [96]. As mentioned above, a prototype strain of RHDV was shown to attach to an α1,2fucosylated carbohydrate structure present on rabbits upper airway and gut epithelial cells [55]. This animal/virus pair could thus provide a natural model to test the co-evolution scenario that we propose.

13. Protection of the newborn by human milk

Human milk contains large amounts of complex oligosaccharides and glycoconjugates terminated with HBGAs epitopes and the oligosaccharides are not degraded in the infant digestive tract since they are recovered almost quantitatively in feces [97]. We and others found that milk from secretor-positive women inhibits the binding of NV VLPs to their carbohydrate ligand, unlike cow milk or milk from *FUT2*−/− women, suggesting that HBGAs from milk oligosaccharides or glycoconjugates could function as decoy receptors to the virus [56,98,99]. In accordance with this hypothesis a recent epidemiological study indicated that breast-feeding protection from norovirus gastroenteritis was most efficient when the maternal milk was rich in α1,2fucosylated oligosaccharides [100]. Since the presence of such oligosaccharides in milk is largely under control of the *FUT2* gene [101], the *in vitro* inhibition data fit with the epidemiological observation. *In vitro* assays showed that the free oligosaccharides are not inhibiting. The milk inhibitors are glycoproteins that correspond to the bile salt-stimulated lipase and to a fraction associating mucins MUC1 and MUC4 [99]. These three glycoproteins present tandemly repeated O-glycosylated sequences that should allow multimeric presentation of the carbohydrate epitopes thus increasing the avidity as frequently encountered in carbohydrate/protein interactions.

In addition to containing α1,2fucosylated structures under control of the *FUT2* gene polymorphism, human milk contains α1,3 and α1,4fucosylated oligosaccharides or glycans partly under control of the *FUT3* gene polymorphism [101]. Yet, irrespective of the ABO phenotype of the mother, it lacks oligosaccharides with A or B antigens. Jiang et al. recently performed a study of the inhibition of the attachment of VLPs from different strains to their distinct carbohydrate ligands [98]. They observed a concordance between the mothers' Secretor (FUT2) and Lewis (FUT3) phenotype and their milk spectrum of inhibition. This strongly suggests that depending upon the combined mother and children *FUT2* and *FUT3* genotypes and upon the virus strain encountered, children may or may not be protected by their mothers' milk. Thus, if a *FUT2*+/− child breast-fed by a *FUT2*−/− mother encounters a virus that binds to α1,2fucosylated carbohydrate ligands, he or she will not be protected by milk decoy receptors. Similarly, irrespective of the mother or the infant ABO blood group, if the child encounters a norovirus strain that binds specifically to either A or B epitopes, such as types 4, 5 and 6 strains of Fig. 7A, breast-feeding will afford no protection by this mechanism due to the lack of appropriate decoy receptors in milk. Therefore, the presence of decoy receptors in human milk and the documented protection that breast-feeding provides, suggest an additional layer of coevolution between noroviruses and us. Strains such as types 1 and 2 of Fig. 7A, which target the largest fraction of the population, are those that will be most frequently subject to the innate protection of newborns by milk decoy receptors. Inversely, strains that target smaller fractions of the population are less likely subject to this protection mechanism, possibly explaining why such strains are still circulating.

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