Blood groups in growth and infectious disease

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Abstract

The human ABO(H) blood group phenotypes arise from the evolutionarily oldest genetic system found in primate populations. While the blood group antigen A is considered the ancestral primordial structure, under the selective pressure of life-threatening diseases blood group O(H) came to dominate as the most common blood group worldwide. Non-O(H) phenotypes demonstrate impaired formation of adaptive and innate immunoglobulin specificities due to clonal selection and phenotype formation in plasma proteins. Compared with individuals with blood group O(H), blood group A individuals not only have a significantly higher risk of developing certain types of cancer but also exhibit high susceptibility to malaria tropica or infection by *Plasmodium falciparum*. The phenotype-determining blood group A glycotransferase(s), affecting the levels of anti-A/Tn cross-reactive immunoglobulins in phenotypic glycosidic accommodation, might also mediate adhesion and entry of the parasite to host cells via trans-species *O*-GalNAc glycosylation of abundantly expressed serine residues, arising throughout the parasite's life cycle, while excluding the possibility of antibody formation against the resulting hybrid antigen. In contrast, human blood group O(H), lacking this enzyme, is indicated to confer a survival advantage regarding the overall risk of developing cancer, and individuals with this blood group rarely develop life-threatening infections involving evolutionarily selective malaria strains.

Introduction

The human gut microbiome has been a subject of comprehensive research for decades, and numerous reviews have discussed various aspects of host-microbe interactions in the context of health and disease. In terms of host genetics and immunity[1, 2, 3] "naturally occurring" immunoglobulins arise from various sources via different molecular pathways, and the ABO blood group is among the genetically determined host factors that modulate the composition of the human intestinal microbiota.[4] Specifically, the microbiota of blood group B individuals differs from those of individuals with non-B antigen phenotypes, for example, showing higher diversity in the *Eubacterium rectale* and *Clostridium leptum* groups. However, when the adaptive production of anti-blood group B-reactive immunoglobulins, which occurs in White Leghorn chickens fed a diet containing *E. coli* O86:B7 lipopolysaccharide,[5] was demonstrated for the first time to occur spontaneously in humans,[6] this form of isoagglutinin production was documented exclusively for the histo (blood) group O(H). Bacterial endotoxins non-specifically stimulate the formation of all immunoglobulins, but prokaryotic blood group A/B-like antigenic structures appear to induce cross-reactive anti-A/B immunoglobulin G[7, 8, 9] that arise in neither blood group A nor B individuals but occur predominantly or exclusively in blood group O(H) due to clonal selection. Consequently, using the non-parametric Wilcoxon signed-rank test in patients suffering from ulcerative colitis, causing increased enteral absorption, the minimally and likely non-specifically elevated levels of anti-B-reactive 7S (IgG) and 19S (IgM) in blood group A plasma were found to remain within the normal range; additionally, a statistically significant increase in anti-B-reactive IgG and IgM immunoglobulins involving less-pronounced, asymmetrically cross-reactive anti-A-specific IgG was detected exclusively in blood group O(H) plasma, and the IgG/IgM quotients showed predominance of the IgG class (Fig. 1). These early, limited observations are consistent with later investigations performed with current experimental tools. For example, Stussi *et al*.(2005)[10] detected anti-A/B cross-reactive IgG in 89% of blood group O(H) sera and anti-B-reactive IgG in 4% of blood group A sera, and vaccination with pneumococcal polysaccharides exclusively elicited anti-A/B cross-reactive IgG but did not affect pre-existing anti-A/B-reactive IgM levels.[11] While the danger theory[12] suggests that there is no adaptive immunity without innate immunity, the bulk of human immunity is not acquired during a single human lifetime but is considered to arise predominantly from evolutionary memory and survival mechanisms. Thus, an inborn origin has been postulated to explain isoagglutinin production in

non-O blood groups.[13, 14] In addition to adaptive, cross-reactive anti-A/B production, which is mainly restricted to blood group O(H) individuals, the majority of anti-A/B immunoglobulins, especially the classic complement-binding anti-A and anti-B-reactive isoagglutinins, measured between 22 and 24°C are not controlled by clonal selection and primarily arise independent of any blood group. These immunoglobulins result from a polyreactive, non-immune, germline-encoded IgM molecule that is released after germ cell maturation and cell renewal, and undergoes phenotype-specific glycosidic accommodation of plasma proteins in the non-O blood groups.[13, 15] It is proposed that depending on the quality of glycosylation, the degree of glycosylation of an immunoglobulin is inversely proportional to its reactivity. While any phenotype-directed auto-reactivity, primarily exerted by germline encoded antibodies, may automatically be neutralized during the course of phenotype formation in normal condition, the dynamic glycosylation of immunoglobulins assumingly plays a key role in physiology and mainly occurs via *N*-linkages. Nevertheless, a proteomics analysis of *O*-GalNAc glycosylation in human serum identified 407 intact *O*-GalNAc glycopeptides from 93 glycoproteins[16]. Thus, in blood group A and B individuals, phenotype-determining GalNAc- or D-Gal glycosylations of plasma proteins are, aside from prevailing *N*-glycosylations, hypothetically associated with mucin-type formation, utilizing *O*-linkages to functional serine/threonine or tyrosine residues from the Fc region of the ancestral IgM molecule[17]. The terminal serine appears to be the crucial structure,[18] and any reduction or exclusion of IgM anti-self reactivity thereby achieved necessarily impairs adaptive and innate defense activities.[15] The key molecule in this phenotypic accommodation might be α2-macroglobulin, which is considered to be an evolutionarily conserved arm of the innate immune system.[19] α2-Macroglobulin is functionally strongly connected to the structurally related IgM molecule, as becomes evident from adhesion of the parasite *Plasmodium falciparum* (*P. falciparum*) to the host cell surface in severe malaria disease,[20] and exhibits ABO(H) blood group reactivity in strict correlation with the cell surface.[21, 22] Consequently, in blood group O(H) individuals, the non-immune and adaptive anti-A (always with Tn cross-reactive) IgM/IgG[15] levels remain unaffected and are still involved in internal and external immune defense. Moreover, the binding of non-immune IgM to the trans-species, blood group A-like or Tn (T-nouvelle) antigen (GalNAc-1-O-Ser/Thr)[23] and to the B-like or T (Thomsen-Friedenreich) antigen (Gal-1-3GalNAc-1-*O*-Ser/Thr)[24], might initiate a secondary response involving the formation of anti-A/Tn and B/T IgG activities, with T cell and natural killer (NK) cell activation,[25, 26] which in the non-O blood groups A, B and AB is affected by glycosidic, phenotypic accom-

modation. This appears to be a general principle that not only may contribute to the increased susceptibility of non-O blood groups to cancer development but even explains the varying susceptibilities of the different ABO(H) phenotypes to special infectious diseases via trans-species molecular complementarity.

Molecular relationship of human ABO(H) blood group phenotype evolution and development to growth and malaria infections

Clonal selection and the principle of glycosidic, phenotypic accommodation explained above have been postulated to contribute to the increased risk of human blood group A individuals developing different types of cancer,[15] and the naturally occurring anti-A-reactive isoagglutinins and anti-Tn-cross-reactive antibodies clearly interact with tumor-associated *O*-glycan structures produced in pancreatic cancer.[27] In a recent cancer risk cohort study, Sun *et al*.(2015)[28] described a statistically significant risk of stomach cancer and pancreatic cancer in blood group A individuals compared with blood group O(H) individuals, while kidney cancer risk was inversely associated with blood type AB. A comprehensive study by Vasan *et al*.(2016)[29] showed positive associations in blood group A individuals with cancer of the pancreas, breast, salivary glands, mouth, stomach and chronic lymphatic leukemia and in blood group B individuals with cancer of the corpus uteri and the bladder; however, inverse associations were observed in blood group A individuals for pharyngeal cancer, esophageal adenocarcinoma, and small intestinal cancer and in blood group B individuals for pleural mesothelioma and myelomas. In two smaller studies, regarding the O/O and A/A genotypes, the above positive associations became even clearer in both cancer of the stomach[30] and that of the pancreas[31]and were finally considered to be established. Thus, blood group A individuals might be burdened with an overall increased risk of developing cancer compared with individuals exhibiting blood group O(H), which is currently thought to confer a survival advantage compared with non-O blood groups.[32, 33].

Considering that a species barrier may be overcome by interspecies glycosylations[34] or "pathogen mimicry", and ABO(H) glycotransferases accomplish the cross-species transmission of *O*-glycans in infectious diseases[35], the proposed principle of glycosidic, phenotypic accommodation may explain the pronounced susceptibility of non-O blood group individuals to the life-threatening infections characteristic of classic malaria tropica, which is caused by *P. falciparum*[36]. While malaria tertiana or infection by *P. vivax* is the best-documented type of malaria[37], *P. falciparum* causes the most severe and frequently encountered type of the disease.

The susceptibility of non-O blood group individuals to the infection by *Plasmodium falciparum* resides in molecular complementarity between the host and parasite

According to a new coalescence analysis performed by Yamamoto et al. (2014)[38], blood group antigen A was again confirmed as the primordial blood group structure and comes first in the evolution. After millions of years, this group mutated in B, while a human specific diagnostic deletion for blood group O (O01) haplotype) was found in Neandertal individuals[39], and finally blood group O(H) became the most common blood group worldwide. There are many possible explanations for this phenomenon. Over millions of years an internal selection process might occur via a currently discussed survival advantage of the overall risk of developing cancer and silent ABO(H) incompatible pregnancies, almost restricted to the blood group O(H), in which maternal IgG antibodies pass through the placenta to the fetal circulation, and cause more or less silent abortions. However, in view of the global distribution of the ABO(H) blood groups, an evolutionarily-selective infectious disease appears to be the major reason. The evolution of the human most likely occurred in Africa[40], where infections by *P. falciparum* or malaria tropica are endemic, while the non-O blood groups A, B and AB are highly susceptible to these infections, with the blood group A appearing to be more affected by the life-threatening form of this disease than blood group B but due to the available numbers is also best studied and compared with blood group O(H)[41]. Consequently, blood group O(H) came to dominate, and its current global distribution is consistent with a selection pressure by *P. falciparum* in favor of group O(H) individuals, which have a survival advantage in malaria-endemic regions.[42, 43] Thus, malaria tropica is currently discussed to contribute to the varying global distribution of ABO(H) blood groups in the human population.[44] Infection by this parasite occurs mainly in tropical and subtropical areas of South America, Africa, and south-east Asia,[45] although episodes of global distribution, involving life-threatening diseases, are a widely discussed problem.[46] Statistically significant life-threatening infections are diagnosed in blood group A[47], B and AB[48] individuals compared with those with blood group O(H), although in areas where this type of malaria is endemic, blood group O(H) individuals may represent the largest group of gametocyte carriers, who sometimes even suffer from mild disease.[43] *P. falciparum* differs from the other human malaria species in that infected red blood cells (RBCs) do not remain in the circulation for the entire life cycle; instead, when young parasites mature to the trophozoite stage, infected RBCs adhere to endothelial cells in the microcirculation.[49] This phenomenon, termed "sequestration", appears to be an-

other characteristic of life-threatening disease resulting from *P. falciparum* infection, in addition to RBC rosette formation. The parasite binds by the PfEMP1 protein (Plasmodium falciparum Erythrocyte Membrane Protein 1)[50] to RBC surfaces via the common *Duffy* Binding-*Like* (DBL) domain, and binds statistically significantly increased to the RBCs of non-O blood groups[41] when compared with those of group O(H). However, because the RBC membrane lacks detectable expression of the blood group A- and B-determining transferases, it is assumed that the binding between host and pathogen is basically accomplished by plasma proteins and soluble transferring enzymes. Indeed, soluble transferases, which accomplish the formation of soluble blood group A/B structures and phenotype formation of plasma proteins[15] (and occurring independently of the secretor status[51, 52]) may also perform the attachment of the inoculated plasmodium sporozoites to the cell membranes of the host. A functional soluble blood group A-transferring serum transferase has been described by Nagai et al. (1978)[53], who transferred UDP-GalNAc to blood group O RBC in *vitro.* Although a transfer of this molecule to a pathogen has never been reported before, the coincidence of statistically significant occurrence of life-threatening infections by *P. falciparum*, with the synthesis of blood group A and B-determining transferases in non-blood group O populations, highly suggest that trans-species glycosylation of blood group A and B glycans plays a key role in this disease. Breaking the species barrier, trans-glycosylation and trans-peptisation may be fundamental in the development of infectious diseases. According to Varki and Lowe (2009)[34], viruses are performing the glycosylations of their proteins by utilizing the host cell machinery, and pathogens invading multicellular animals may decorate themselves with structures that appear to be identical to those found on their host cell surfaces; Kreisman et al. (2012)[54] argued that a pathogen seeking to bind to cell membranes of the host may first encounter the specific glycans attached to soluble mucinous structures. In fact, while mucin-type *O*-GalNAc glycosylation likely plays a major role in cell adhesions[55], soluble blood group A and B-determining enzymes hypothetically initiate the synthesis of adhesion molecules via heterologous mucin-type *O*-glycosylation of the parasite's serine repeat antigen (SERA); that is, a serine-rich protein providing numerous *N*- and *O*-glycosidic sites. This protein primarily appears during the intraerythrocytic stages of the parasite, while serine residues are expressed at all stages of the parasite' life cycle, and even occur on the surface of inoculated sporozoites, which are proteolytically processed by a serine protease.[56] The SERAs are encoded from a multigene protease family, common to all *Plasmodium* species[57, 58] but occurring in different types, from which the serine type SERAs-SERA5 and cysteine type SERA-SERA6 show

the highest levels in *P. falciparum*[59, 60]. According to a recent study, SERA5 has been discussed to have no enzymatic role[61] during the blood-stage growth of *P. falciparum*, and although obviously regulating the kinetics and efficiency of malaria parasite invasion and egress from host erythrocytes, neither SERA5 function nor the role of its processing appear to be completely understood[62]. Nevertheless, in view of the strong susceptibility of the human blood group A organism to infection by *P. falciparum*, SERA5 highly suggests to function as an acceptor in enzyme-substrate competition between host and parasite in mucin-type *O*-GalNAc glycosylations. Again, as viruses are performing the glycosylations of their proteins by utilizing the host cell machinery[34], plasmodium species may utilize the same method (Fig. 2). Without questioning the established role of RIFINs,[44, 63, 64, 65] which are discussed to be provided by the pathogen alone, a functional blood group A-determining plasma transferase transferring the blood group A glycan, may indicate an additional and/or more complex pathomechanism, involving the trans-species A-like, Tn-mucin-type GalNAc-1-*O*-Ser/Thr-R structure[23], representing a classical adhesion molecule that promotes glycan elongations and the binding between parasite and host. Finally, the human blood group A-specific α1-3-N-acetylgalactosaminyltransferase and blood group B-specific α1-3-galactosyltransferase, expressed by plasma proteins might, together with serine/threonine kinases produced by *P. falciparum*, provide the essential metabolic condition for trans-species synthesis of mucin-type adhesion proteins and GalNAc-1-O-Ser/Thr or Tn[23] glycosylation in blood group A, and Gal-1-3GalNAc-1-*O*-Ser/Thr or T[24] glycosylation in the blood group B. This proposed glycosidic accommodation of plasma proteins[14, 15] is not a lock-and-key event but a dynamic process, dominated by continuous synthesis of α2-macroglobulin and its functional synergism with the structurally related non-immune IgM; this molecule being engaged in the adhesion process via its Fc region[17, 66] and *O*-glycosylation[14, 15] of germline-serine-residues,[18] obviously cannot induce rosetting on its own,[20] whereas α2-macroglobulin crosslinks multiple PfEMP1 molecules and, thus, is an integral component of rosette formation. The differences in the life cycle of the parasite during uncomplicated malaria in blood group O(H) individuals and life-threatening disease in blood group A and B individuals remain unknown. Again, the complex infection process is presumably initiated by heterologous glycosylation, which has long been considered the major carbohydrate modification in the intraerythrocytic stage of *P. falciparum*,[67] and *O*-glycosylation appears to be the major form,[68] whereas the role of the ABO(H) phenotypes has largely been ignored. The metazoan/eukaryotic lineage, which comprises the evolutionarily first protein glycosylation by GalNAc via *O*-linkages on

peptides displaying serine/threonine motifs, is characterized by mammalian embryonic stem cell fidelity to initiate and complete germ cell maturation. When breaking the species barrier, this glycosylation may promote the asexual reproduction of *Plasmodium* merozoites, which involves immature gamete formation, and may even explain the attachment of infected to uninfected RBCs. Indeed, rosette formation occurs predominantly with human blood group A RBCs, [41, 47] while incomplete formation in blood group O(H) RBCs, as molecularly explained by Moll et al. (1915),[69] may be associated with weak disease. Serine/threonine and tyrosine kinases are exported by *P. falciparum*,[70, 71] and the infected RBC membrane exerts serine transferase activity, representing a potential anti-malaria drug target during the asexual intraerythrocytic stage.[71, 72] Thus, SERA, which was detected decades ago,[73] was accepted as a putative antigen precursor, while heterologous *O*-GalNAc glycosylation, which most likely utilizes trans-species-compatible serine positions on the surface of plasmodium sporozoites, might explain the preferred binding of special *Plasmodium* strains. In conclusion, the pronounced susceptibility of blood group A individuals to severe malaria[36] suggests the formation of a hybrid Tn-reactive structure, providing another molecular definition of a potential immunological (therapeutic) target, while blood group O(H) individuals, who are unable to complete this self-destructive, likely transient, hybrid connection further maintain both natural non-immune anti-A/Tn IgM and IgG cross-reactive antibodies against this deleterious connection (Fig. 2 and 3).[15] In blood group B, the adhesion of plasmodia to RBCs occurs via Gal-1-3GalNAc-1-*O*-Ser/Thr or T[24] glycosylation, which additionally acts also in blood group AB. In the blood group O(H) phenotype, incomplete adhesions may arise via non-specific, blood group A- and B-independent glycans, while analyses of AO exons and introns in individuals with an "A-to-weak B phenotype" have revealed a novel O1v-A2 hybrid allele leading to missense mutations in A-transferases and/or transferees[74]. Since using the blood type molecular testing and genotyping in transfusion medicine, the number of weak ABO(H) alleles is still growing.[75] Unusual O alleles, including O2, at the ABO(H) locus may be implicated in unexpected blood group phenotypes,[74] whereas the O(H) phenotype is no longer considered a genetic entity.[76, 77] It would be interesting to study the binding of *P. falciparum* to weak A alleles that appear to serologists as group O but produce "irregular" anti-A_1, whose reactivity with Tn-structures is unknown and also arises in some individuals with blood group A_2,[78] which most likely results from the lack of GalNAc transferase A_1 and exclusive encoding of a specific GalNAc transferase A_2. This human-intrinsic enzyme is genetically distinct from A_1 transferase.[79, 80, 81] Furthermore, in view of the more recent experi-

ments by Blixt et al. (2012),[82] the chemical simplicity of the Tn antigen does not necessarily stand for a simple antigenicity, which changes with various peptide backbones. Finally, blood type molecular and genotyping studies on malaria patients with weak A alleles in connection with non-immune, natural anti-A/Tn levels potentially provide even more insight into the actual trend of ABO(H) blood group evolution.

Induction of infectious diseases occurs via molecular complementarity between host and pathogen

The process of *P. falciparum* merozoite invasion, most likely involving SERA5, shows an intriguing connection to early metazoan growth processes, which appear to be dominated by trans-species *O*-GalNAc1-*O*-Ser/Thr-R or Tn glycosylation.[83, 84] Under normal conditions, this glycosylation is followed by rapid deglycosylation and may further be followed by the specific glycosidic construction of the A, B and AB phenotypes in humans[15] after zygote formation, with the exception of blood group O(H). These phenotypes arise from the evolutionarily oldest genetic system found in primate populations[85] but always occur in critical complementarity with the metabolism and recognition tools of distinct eukaryotic and prokaryotic pathogens. The species-independent ABO gene polymorphism has been extensively analyzed in primates,[80, 86] but an understanding of the ramifications of this polymorphism has been elusive.[87] While the pronounced susceptibility of blood group A individuals to the infection by the eukaryote *P. falciparum* appears to be established, moreover, molecularly is explainable through different concepts, reports describing the susceptibility of different ABO(H) blood groups to prokaryotic, viral and bacterial infections are more controversial.[88,89, 90] Although the phenomenon of pathogen mimicry is known for decades, there are few examples by which the complementarity between the phenotype-determining enzymes of the host and the acceptor of the pathogen becomes molecularly evident. Focusing on the blood group O and its discussed susceptibility to infection by *Helicobacter pylori* (*H.pylori*) and special strains of *Vibrio cholera* (*V. cholera*), reveals that the genetic, metabolic and immunological relationships between host and pathogen are much more complex than those between the non-O-blood groups and the eukaryotic parasite *Plasmodium falciparum* or malaria tropica disease. Whereas intestinal fucose functions as a mediator of host–microbe, hybrid symbiosis [91], the infections by *H.pylori* and *V. cholera* occur on the epithelial barrier of the body under completely different topographical conditions when compared with malaria.

According to current reviews,[89, 90, 92] the number of patients with blood type O(H), which apparently develop more frequently *H. pylori* infections, does not differ statistically significant from that, in which the ABO blood type does not play any role when compared by culture results. Rossez et al (2012)[93] have characterized the glycosylation pattern of gastric mucins and showed that about 80% of *O*-glycans carried A, B or H antigens and 30 to 50% expressed H and/or Le[b] epitopes, which occurred most pronounced in blood group O individuals, whereas Boren et al. (1993)[94] have shown by thin-layer chromatography that *H. pylori* predominantly recognize Le[b] and H-active structures. Such observations have suggested that *H.pylori* infections might primarily be linked to the secretor status and that non-secretors (Le[a] + Le[b]-) are more susceptible. However, independent of *H. pylori* infections, individuals with blood group O and/or non-secretors are especially prone *per se* to inflammatory bowel disease and gastric or duodenal ulcers but show more severe symptoms during these infections, whereas a variety of other factors are responsible for *H.pylori* virulence, as summarized in the current review by Kao et al, (2016);[92] *H. pylori* infection alone may be asympomatic for a long time. Intriguingly, α1,2 fucosyltransferase, which represents the basis for the synthesis of all ABO(H) epitopes and is secreted independently of the secretor status,[51, 52] will also be produced by *H. pylori*. Thus, both host and pathogen produce the critical enzyme, and it is not clear whether pathogen adhesion is initiated by the host or the pathogen.

Although α1,2-fucosyltransferase is produced by *H. pylori* and other Enterobacteriaceae,[95] corresponding enzyme activity does not appear to occur in *V. cholera* strains and has not been reported for either the *V. cholera* El Tor or the *V. cholera* O139 strain, showing the strongest virulence and develop the most severe disease in human infections. The first studies that suggested a relation between cholera and ABO(H) blood groups were performed by Barua & Paguio (1977)[96], who reported a pronounced susceptibility to this infection in individuals with blood group O. This observation was substantiated by subsequent studies [97], while according to other reports one third of cholera patients are non-secretors[98, 99], which is highly significant because the number of non-secretors in all populations is not more than about 20 percent. However, blood group O does not increase the risk of being infected with *Vibrio cholera*:[100] when ABO phenotypes were compared by culture results, the groups O and A were equivalent in *Vibrio*-positive and *Vibrio*-negative patients, but when the effect of cholera toxin (Ctx) was studied on enteroids, a significantly greater cyclic adenosine monophosphate response was shown in enteroids, which were derived from blood group O stem cells[101]. Thus, in case of infections, individuals with

group O develop more frequently life-threatening disease and are more likely to be hospitalized with severe cholera[102] when compared with non-O blood groups[103].

While in malaria tropica and blood group A individual the parasite's entry into the patient's cell is probably determined via the functions of the soluble blood group A phenotype-determining enzyme(s), the entry of the cholera toxin into the cytosol of blood group O individuals is explained by a different mechanism. Over the decades several models have been developed that try to explain the adhesion of pathogenic Enterobacteriaceae or endotoxins to mammalian cell membranes. As early as 1947 Burnet and Stone[104] discovered the *"the receptor-destroying enzyme"*, a mixture of hydrolases dominated by a neuraminidase, associated with the secretion of adhesion proteins[105] that cause pan-agglutination and RBC rosette formation,[106] which is known as the Hübener-Thomsen Friedenreich phenomenon.[107] The adherence of *V. cholerae* to isolated rabbit brush border membranes[105] of human RBCs and the lymphoid follicle epithelium of the intestine was (depending on the cellular HA type) inhibited by L-fucose.[108] These observations fit the established concepts. The principle is comprehensively described by Cooling (2015)[109] and explained through a graphic, according to which the adhesion is thought to be initiated by the pathogen, utilizing preformed fucosylated structures of the host, while the adhesion of the Ctx holotoxin occurs via a complex binding process between the five toxin B subunits and one A subunit to cell surface receptors in the small intestine[110]. Nevertheless, this graphic gives rise to speculation regarding a functional contribution of the host and suggests an adhesion that might be accomplished by soluble α-1,2-L-fucosyltransferases of the host via *O*-linkages and creation of a mucin-type adhesion molecule, utilizing hydrophilic amino acids (serine/threonine residues),[111] provided by the Ctx B and/or A subunits, which get attached to the ganglioside GM1, Galβ1-3GalNAcβ1-4Galβ1-4Glc-ceramide. Again, the production and levels of soluble ABO(H)-determining glycotransferases are independent of the secretor status,[51, 52, 112] and it may be hypothesized that a host-provided adhesion potentially occurs as well, in which the pathogen utilizes the host cell machinery. This hypothetical α1,2-L-fucosylation occurs independently of the ABO(H) blood group and secretor status, and in the non-O blood groups A and B, type O(H) or H antigenicity is reduced or neutralized by subsequent phenotypic A and B glycosylation that occupies fucosyl residues, whereas the blood group O(H) individual will neither exhibit a natural, non-immune anti-H antibody against its own epitope under normal condition, nor produce an adaptive anti-H-reactive antibody due to clonal selection (Fig. 3). Significant production of adaptive and innate anti-H antibodies is limited to the extremely rare, classical O(h) *Bombay*-type in-

dividual, lacking the normal, human-specific FUT1/FUT2 fucosylations[113] and respective fuco-sylations of plasma proteins[114]. At present, the lowest prevalence of blood group O(H) world-wide occurs in the Ganges delta, where cholera has been endemic for centuries.[115, 116] However, the evolutionary-selective pressure, which is exerted by the eukaryote *P. falciparum* and malaria tropica infections on blood group A populations, is obviously much stronger. Consequently, blood group O(H) offering the widest flexibility in adaptive and germline-encoded immunity, has survived as the most frequently occurring blood group worldwide[117] despite extensive historical cholera pandemics.[118, 119, 120]
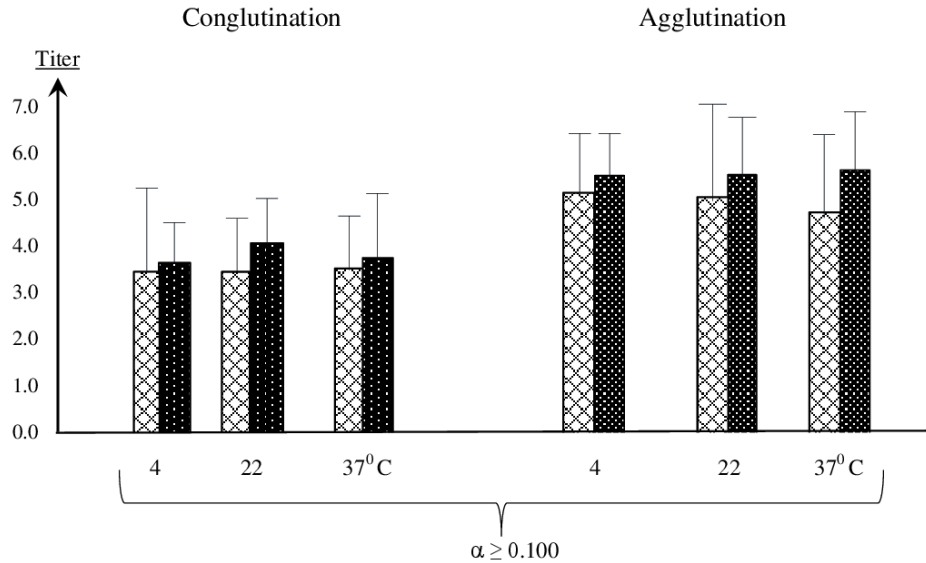
Conclusion

"Naturally occurring" immunoglobulins arise from various sources via different molecular path-ways.[8] While clonal selection protects mammalian species from the formation of environmentally induced adaptive, self-reactive immunoglobulins, the human blood group A, B and O(H) pheno-types additionally exclude the presence of a self-reactive, germline-encoded non-immune IgM through identical glycosylation of cell surfaces and plasma proteins.[14,15] Consequently, pheno-type-specific susceptibility to infection by a special pathogen necessarily reduces or excludes natural immunological protection (Fig. 2). This implies that the phenotype-determining glyco-transferase might play the first role in determining the susceptibility of an individual to infection by a special pathogen; this enzyme both establishes contact with a complementary acceptor of the pathogen and reduces or excludes the presence of a phenotype-specific antibody through glyco-sidic exclusion and/or accommodation of plasma proteins.[14,15] Again, the observations accumu-lated in the literature suggest that an infectious disease may be initiated via phenotypic accom-modation between host and pathogen. In both the infection by the eukaryote *P. falciparum* and the prokaryote *V. cholera,* the pathogen adhesion may be initiated by trans-glycosylation and binding of the phenotype-determining carbohydrate (GalNAc and/or L-fucose) on hydrophilic amino acids (serine/threonine) from the pathogen peptide via *O*-linkages. Thus, in the case of se-vere malaria in individuals with human blood group A, or of cholera disease in blood group O, the contact between host and pathogen appears to be initiated through "destructive cooperation" between heterologous-complementary carbohydrates and peptides. The anti-ABO(H)-reactive immunoglobulins remain basically engaged in the control of those internal growth processes, from which most of them arise. Nevertheless, these immunoglobulins may non-specifically or cross-reactively protect the organism from environmental infections and pathogens that are not

involved in their production, a phenomenon characteristic of the polyreactive defense proteins of invertebrates. The anti-A/anti-Tn-cross-reactive hemagglutinin emerging from the coat proteins of fertilized eggs of *Helix pomatia*[15] and lectins from other snails agglutinate *Staphylococcus aureus, Escherichia coli, Listeria* and several *Salmonella* species. This effect is likely due to *N*-acetyl-D-galactosamine residues[121] because all bacteria utilize this sugar in different metabolic pathways, which do not necessarily lead to complete antigenic structures. Thus, the central immunological position of the human histo (blood) group O(H) remains evident in its evolutionary metabolic cross-point and comprehensive presentation of both nonimmune IgM and adaptive IgM/IgG antibodies. These antibodies are mainly directed against all non-O blood groups, involving their cross-specific developmental and/or "aberrant" structures (Fig. 3), as early metazoan eukaryotic *O*-glycans, Tn [23] and T [24] antigens have been identified. These ancestral glycans arising from *O*-glycosylations, which are used (with similar peptide backbones) by lower metazoans, such as mollusks and the fruit fly *Drosophila melanogaster*[122, 123]*;* moreover, in the snail *Helix pomatia*, they are associated with the release of a hexamerically[124] structured Tn-complementary hemagglutinating defense protein. Hammarström (1973)[125] showed that the binding patterns and the capacity of this molluscan protein to bind human blood group A RBCs are strikingly similar to those of the mammalian IgM molecule, giving rise to speculation regarding an evolutionary relationship with the mammalian non-immune anti-A-reactive IgM molecule. While the germline of *Helix pomatia* appears not to be burdened with A/B phenotypic accommodations, similar to the polyreactive ancestral IgM of human blood group O(H), this species and other snails never develop cancer. An even stronger immunity, involving a clinically relevant anti-H-reactive, complement-binding IgM and most likely higher anti-A/Tn and anti-B/T levels than in blood group O(H), is exerted by the "real" blood group O or *Bombay*-type O(h) (h/h; se/se), according to a mutational meltdown of the H and Se gene functions on chromosome 19 (encoding the α1,2 L-fucosyltransferases (FUT1/FUT2)). However, the extremely small population size of the classic *Bombay* type suggests an effect on reproductive health[114] and demonstrates a negative evolutionary role. While the background of these mutations has been discussed controversially and studies on the functions of α1,2 L-fucosyltransferases in murine fertility[126] may not be extrapolated to human phenotype development, primates use different pathways for critical fucosylation events,[127] and human α1,2 L-fucosyltransferase genes are exclusively responsible for the formation and phenotypic expression of ABO(H) antigens[128]. Thus, the formation of the (Oh) Bombay type represents the evolutionary opposite to that of the normal blood group (O(H), which fi-
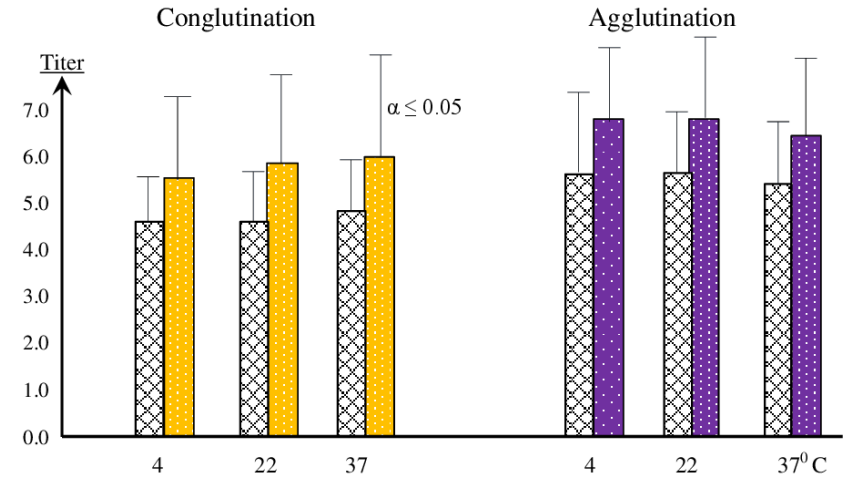
nally became the molecularly and immunologically explainable most common blood group worldwide. Although an internal selection process might, via a currently discussed survival advantage of the overall risk of developing cancer, together with silent ABO(H) incompatible pregnancies, have occurred over millions of years, in view of the global distribution of the ABO(H) blood groups, an evolutionarily-selective infectious disease appears to be the major reason, and the selective pressure exerted by life-threatening malaria tropica, which is deleterious to non-O blood groups, explains the biological predominance of blood group O(H) much more.

Selective immune response of blood group O individuals to increased enteral absorption
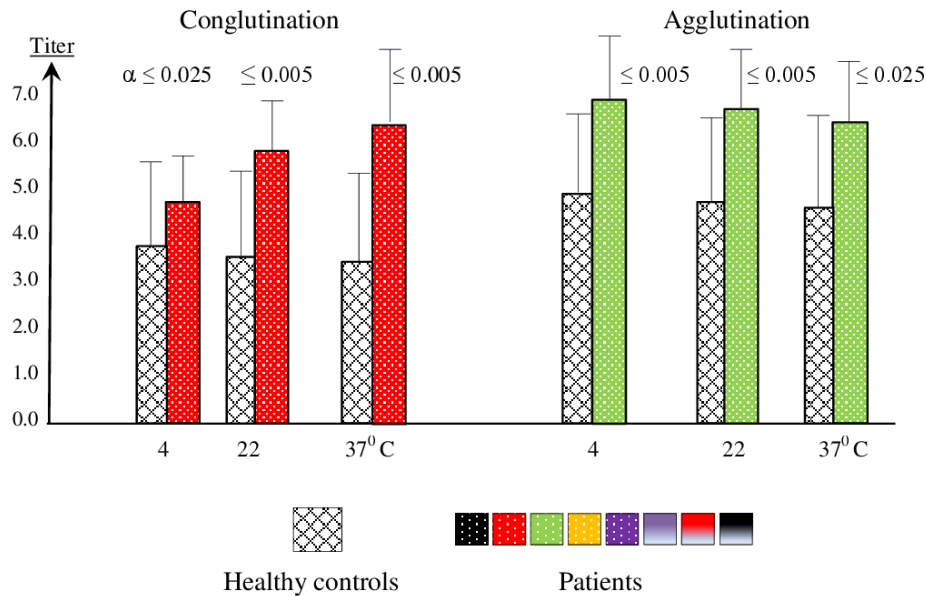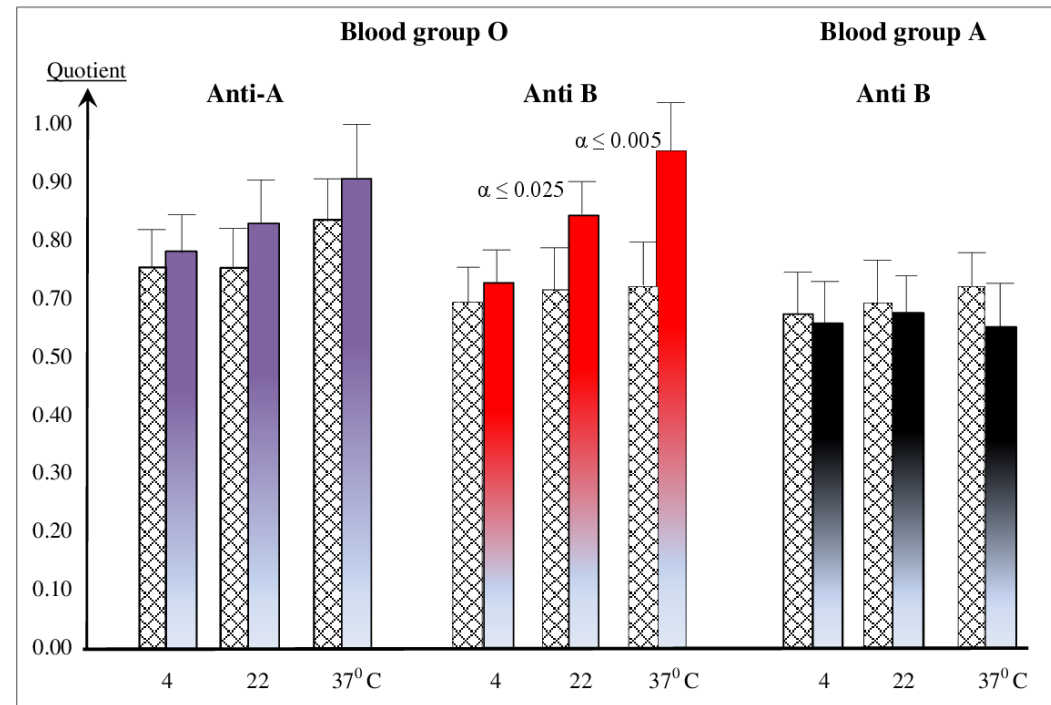
Fig. 1. Spontaneous variations in the production of anti-A/B isoagglutinins in 21 individuals suffering from ulcerative colitis (blood group A = 11, blood group O = 10), and in 42 normal persons (blood group A = 22, blood group O = 20). The grade of immunization and dominance of the 7S (IgG) conglutinating and 19S (IgM) agglutinating immunoglobulins were determined by measuring conglutinin and agglutinin titer and their quotients in triple serum samples at three different temperatures under double-blind conditions. The number of the respective dilution stage was used for the evaluation; this number corresponds to the negative logarithm of base 2 of the dilution. By means of the non-parametric Wilcoxon signed-rank test, the anti-B-reactive IgG and IgM levels in blood group A plasma remained within normal range, whereas the blood group O patients exhibited the statistically significant stimulation of an IgG conglutinating and IgM agglutinating anti-B response as well as an asymmetrically cross-reactive, less pronounced anti-A specific IgG response, and the IgG/IgM quotients indicate the predominance of the IgG class. The figure is reconstructed from the data published in Arend & Fehlhaber (1969)[6].

Molecular mechanisms of infectious diseases
Blood group phenotype-dependent pathogen adhesion
molecularly excludes corresponding antibody formation

Blood group O

Polyreactive

Non-immune IgM-Ser-OH

IgG *secondary response*

Incomplete adhesion

HO-Ser Ser-OH Ser-OH

HO-Ser Ser-OH

-O-Ser

Plasmodium falciparum

GalNAcα1

-O-Ser

Plasma proteins (N-/O-) - *Ser*-O-

Tn-*mucin-type adhesion*

IgG *non response*

GalNAc-T(s)

GalNAcα1

Blood group A

Fig. 2

Fig. 2. Complementarity and adhesion of mammalian structures to a pathogen exclude the formation of adaptive and innate antibody reactivity against the resulting hybrid antigen. The parasite *Plasmodium falciparum* exhibits molecular complementarity to blood A synthesis by serine repeat antigen (SERA), exerting a surplus of serine residues, which may function as acceptor sites on the surface of inoculated sporozoites for soluble blood group A-determining plasma transferase, α1-3-N-GalNAc-T, hypothetically synthesizing a trans-species, hybrid A-like, Tn-mucin-type GalNAc-1-*O*-Ser/Thr-R structure. Blood group A cannot respond by immunoglobulin G (IgG) antibodies to A or A-like cross-reactive Tn structures due to clonal selection, while the non-immune IgM undergoes phenotypic accommodation, moreover, gets involved in the adhesion mechanism by its Fc-region[66] via *O*-GalNAc glycosylation of germline-serine-residues[18]. Individuals with blood group O(H) lacking this enzyme, may not develop this hybrid antigen and maintain the antibodies against it, while the parasite does not completely get attached to the cell membranes of the host.

Central immunological position and evolutionary, metabolic crossover point of the human histo (blood) group O(H) between trans-species A/B-like (Tn/T) and species-intrinsic phenotypic A/B-allelic glycosylations
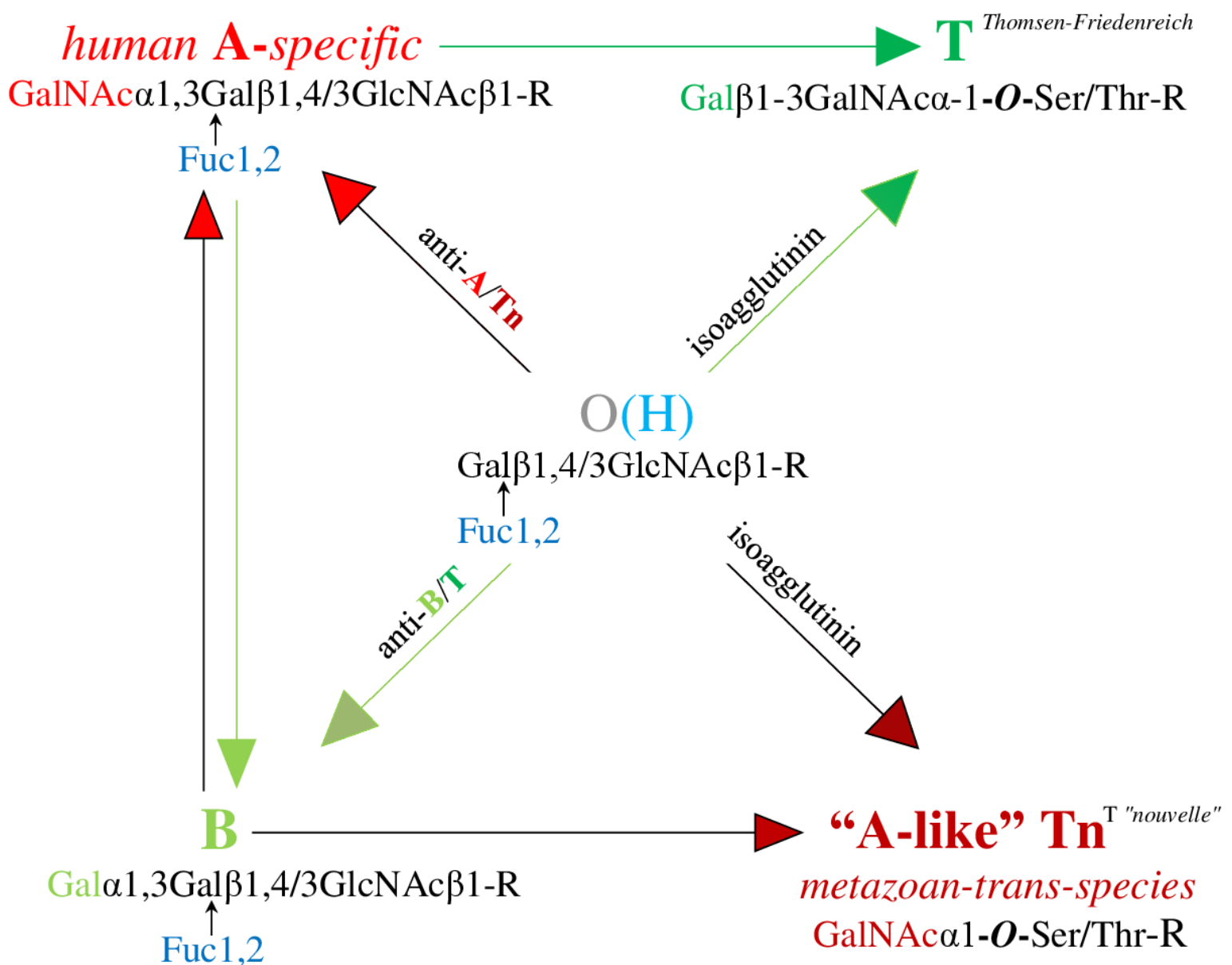

human **A**-*specific*

GalNAcα1,3Galβ1,4/3GlcNAcβ1-R

Fuc1,2

T *Thomsen-Friedenreich*

Galβ1-3GalNAcα-1-***O***-Ser/Thr-R

anti-**A/Tn**

isoagglutinin

O(H)

Galβ1,4/3GlcNAcβ1-R

Fuc1,2

anti-**B/T**

isoagglutinin

B

Galα1,3Galβ1,4/3GlcNAcβ1-R

Fuc1,2

"A-like" Tn T *"nouvelle"*

metazoan-trans-species

GalNAcα1-***O***-Ser/Thr-R

Fig. 3. The central immunological position of blood group O(H) is evident based on its comprehensive presentation of both adaptive and germline-encoded "natural" antibodies against all mature A and B glycans and their cross-reactive developmental structures Tn and T. Human A-specific (A-allelic) glycosylation and trans-species "A-like" Tn formation are developmentally connected via the formation of cross-reactive anti-A/Tn isoagglutinin. According to Hofmann et al.(2014)[27], blood O(H) sera bind to both Tn and T antigens, and the anti-A isoagglutinin levels in blood group O(H) and blood group B sera are associated with an anti-Tn antibody that does not react with blood group B red cells or T glycoconjugates. By contrast, the anti-B antibodies of blood group A sera and blood group O(H) sera bind to B and T glycoconjugates but not to A or Tn glycoconjugates. This figure was constructed according to Arend (2017).[15]

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