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Proposed *O*-GalNAc/Gal glycosylation pathways in blood group O and non-O blood groups during *Plasmodium falciparum* infections

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Abstract

The coevolution of species drives diversity in animals and plants and contributes to natural selection, while in host–parasite coevolution, a parasite may complete an incomplete evolutionary/developmental function by utilizing the host cell's machinery. Analysis of related older data suggests that *Plasmodium falciparum* (*P. falciparum*), the pathogen of malaria tropica, cannot survive outside its human host because it is unable to perform the evolutionarily first protein glycosylation or blood group-independent (serologically A-like) *O*-GalNAcα1-Ser/Thr-R, Tn antigen ("T nouvelle") formation owing to its inability for synthesizing the amino sugar *N*-acetyl-D-galactosamine (GalNAc). This parasite breaks the species barrier via hijacking the host's A-like/Tn formation by abundantly expressing serine residues and creating hybrid A-like/Tn structures. In the human blood group O(H), these hybrid structures are attacked by the germline-encoded nonimmune polyreactive immunoglobulin M (IgM), which physiologically regulates the expression of the syngeneic A-like/Tn antigen. In non-O blood groups, this antibody molecule has undergone the phenotypic accommodation of plasma proteins, which results in loss of blood group A- and B-corresponding anti-A and anti-B isoagglutinin activities. This loss allows the generation of human A- and B allele-connected hybrid epitopes and the development of life-threatening disease almost exclusively in non-O blood groups. Although malaria infection occurs regardless of the blood group, the synthesis of the blood group AB enables the strongest contact with the pathogen, and molecularly precluding any isoagglutinin activity makes this group the least protected and the smallest among the ABO blood groups. In contrast, blood group O(H) individuals have the least contact with the pathogen; they maintain the isoagglutinins, rarely develop severe disease, and survive this coevolution in an immunological balance with the pathogen as the largest blood group worldwide.

Keywords: trans-species *O*-glycosylation; trans-species functional bridge; phenotype-specific plasma glycosylation; glycosidic exclusion; ontogenetic Tn formation

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Malaria-causing parasites interact with glycoprotein synthesis and form hybrid ABO blood types to complete their life cycle.[1]

In an earlier article, I proposed that in malaria tropica infection the first step of pathogen transmission occurs regardless of the blood group across species barriers via molecular mimicry or formation of an intermediate hybrid Tn antigen [1], and showing another evolutionary function of this developmental structure. Meanwhile, my actual analysis of related older data suggests that the malaria-causing parasite *Plasmodium falciparum*, which cannot survive outside its human host, lacks complete protein synthesis. This protozoan parasite does not perform the metazoan host's evolutionarily first protein glycosylation, or blood group-independent, (serologically A-like) *O*-GalNAcα1-Ser/Thr-R, Tn antigen ("T nouvelle") formation owing to its inability for synthesizing the amino sugar *N*-acetyl-D-galactosamine (GalNAc): although some studies have reported traces of GalNAc in the parasite [2][3], more recent research investigating the cytosol across multiple life cycles has not detected the molecule [4][5][6]. Furthermore, glucosamine (Glc)-GalNAc epimerization does not occur in the parasite [7], nor does it possess genes required for mucin-type *O*-glycan synthesis [8]. When this parasite evokes life-threatening disease predominantly in people with blood group A, the host's proteoglycome plays a significant role in pathogenesis.

In fact, in host–parasite coevolution, parasites may complete their development using host cell machinery, with mucin-type *O*-glycans acting as a trans-species functional, molecular

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Abbreviations: Ab, antibody; IgG, immunoglobulin G; IgM, immunoglobulin M; D-GalNAc, *N*-acetyl-D-galactosamine; D-Gal, D-galactose; FUT1 and FUT2, galactoside 1,2-alpha-L-fucosyltransferase 1 and 2; RBC, red blood cell.

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bridge. The human histo-blood group antigens are potential factors for rotavirus cross-species transmission; in particular, blood group A antigen and A-like GalNAc residues have been identified as receptors [9]. According to a glyco-evasion hypothesis, a pathogen can alter host immune systems by hijacking the glycosylation pathways [10]. Evidence shows that parasitic helminths (worms) coevolve with vertebrate immune systems [11].

The pathogen *P. falciparum*, which is transmitted to human hosts through the anthropophilic mosquito *Anopheles gambiae* [12], does not have the cellular machinery to complete protein glycosylation independently and hypothetically breaks the species barrier by abundantly expressing serine residues [13] for hijacking host glycosylation, specifically the (serologically A-like) *O*-GalNAcα1-Ser/Thr-R or "T nouvelle" (Tn) [14] antigen formation process. Although several glycans can be *O*-linked to Ser/Thr residues, *O*-GalNAc glycans appear to be especially relevant to mucosal sites and other locations of parasitic invasion [15]. The "bulky" [16] polyfunctional GalNAc molecule is a crucial component of glycosylation, an evolutionarily conserved pathway, which dominates critical functions during metazoan growth and reproduction and may even be the target of *P. falciparum* in a recently observed inverse relationship between global malaria incidence and cancer mortality [17].

Metazoan protein *O*-glycosylation and human ABO blood group phenotype formation: A hypothetical basis of *P. falciparum* infection and malaria tropica disease.

In metazoan evolution, up to 20 distinct genetically undefined polypeptide *O*-GalNAc transferases catalyze the first addition of GalNAc to a protein [18] and synthesizes the above (serologically A-like) *O*-GalNAcα1-Ser/Thr-R Tn [14], which results from the most complex and differentially regulated step in protein glycosylation [19] and represents a normal yet fleeting intermediate structure, characterizing stem cell fidelity [20][21]. Hypothetically, it occurs as a

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reversible glycosylation and predetermined breaking point of the human polyreactive nonimmune immunoglobulin M (IgM), whose secretory version represents an *O*-glycan-depleted antibody molecule, exerting serine residues at its V and/or Fc immunoglobulin regions [22][23] and acts as the complementary protein of the A-like/Tn antigen. The primarily pentameric structure of this mammalian antibody gives rise to speculation regarding an evolutionary relationship with the hexameric structure of the anti-A-reactive *Helix pomatia* agglutinin [24], a primitive invertebrate defense protein, emerging from the coat proteins of fertilized eggs and reflecting the snail-intrinsic, reversible *O*-GalNAc glycosylations [25]. The mammalian nonimmune IgM is considered the humoral spearhead of innate immunity, which is not only produced by B cells but also throughout epithelial tissues [26][27] and demonstrates the serological profile of the human blood group O(H) via complement-mediated, cytotoxic anti-A and anti-B isoagglutinin activities [28][29] (Fig. 1).

An X chromosome-encoded T-synthetase appears to control the Tn antigen expression [30] through carbohydrate chain elongation and mucin-type glycoprotein formation [31][32][33], which involves the subsequent synthesis of the disaccharide Galβ1-3GalNAcα1-O-Ser/Thr or "T" antigen (also known as the Thomsen-Friedenreich antigen) [34] and is completed by the ABO phenotype and Lewis-type generation. Intriguingly, serological data obtained as early as 1971 [35], indicated an X chromosome/germline-encoded origin for the murine nonimmune anti-A-reactive IgM, although the complementarity of this antibody protein with syngeneic A-like ovarian glycoproteins and/or glycolipids became evident in subsequent studies [36][37]. Murine and human nonimmune IgM exhibit a serologically similar reaction with human red blood cells (RBCs) and human anti-A and anti-B isoagglutinin activities have been attributed to anti-A/Tn and anti-B/T antibodies owing to their cross-reactivity with Tn and T structures [38]. During the establishment of the human ABO blood group phenotype these innate

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isoagglutinin activities, exerted by the nonimmune IgM, are downregulated through phenotypic glycosidic accommodation of plasma proteins in the non-O blood groups A, B, and AB [28][29]. Because of donor-substrate availability, protein glycosylation is thought to occur intracellularly in the Golgi cisternae, in which nonimmune immunoglobulin production may be adapted to ABO phenotype formation. Accordingly, during immunoglobulin secretion, the poorly glycosylated intracellular IgM molecule becomes loaded with L-fucose and D-galactose [39], and the subsequent expression of fucose and galactose residues by the extracellular IgM might form the basis of phenotypic accommodation. This process may also be performed and/or completed by soluble plasma glycotransferases: blood platelets, for example, have been detected as a rich source of both glycosyltransferases and energy-rich sugar and amino sugars that are released from activated platelets to function in the extracellular space [40] [41] and potentially contribute to the phenotypic accommodation of plasma proteins. A single *O*-glycosidic enzymatic step may create an A, B, or AB mucin-type cell surface epitope and release a secretory IgM, lacking the corresponding anti-A/Tn or anti-B/T isoagglutinin activities, whereas the blood group O(H) maintains these activities.

The lack of any ABO blood group glycosylation or phenotype formation, as shown by the rare O(h) or *Bombay type* [42][43], which originates from consanguinity, is associated with strongly elevated isoagglutinin levels and an unusual anti-H reactivity, acting over a wide range of temperatures, with a thermal amplitude at 37 °C. This overexpression of anti-glycan antibody activity might cause autoimmunological impairment of germ cell maturation or function, responsible for the male infertility of this group [43]. In contrast, the blood group AB constitutes (likely to a result of evolutionary selective diseases) the smallest among the human ABO blood groups, precluding any isoagglutinin formation and representing the other extreme of phenotype diversity. Thus, the O(h) or *Bombay type* and blood group AB appear to mark two opposite

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directions of negative (natural) selection and demonstrate how phenotype and isoagglutinin production form evolutionary functional unity, wherein the degree of phenotype diversity and innate immunity are inversely proportional and show again the central evolutionary and immunological position of the human blood group O(H) [29][1] (Fig. 2).

The pathogenesis of malaria will be discussed in view of these complex developmental and evolutionary/immunological conditions, under which *P. falciparum* reaches every tissue during its life cycle progression and accesses the proteoglycome of the host.

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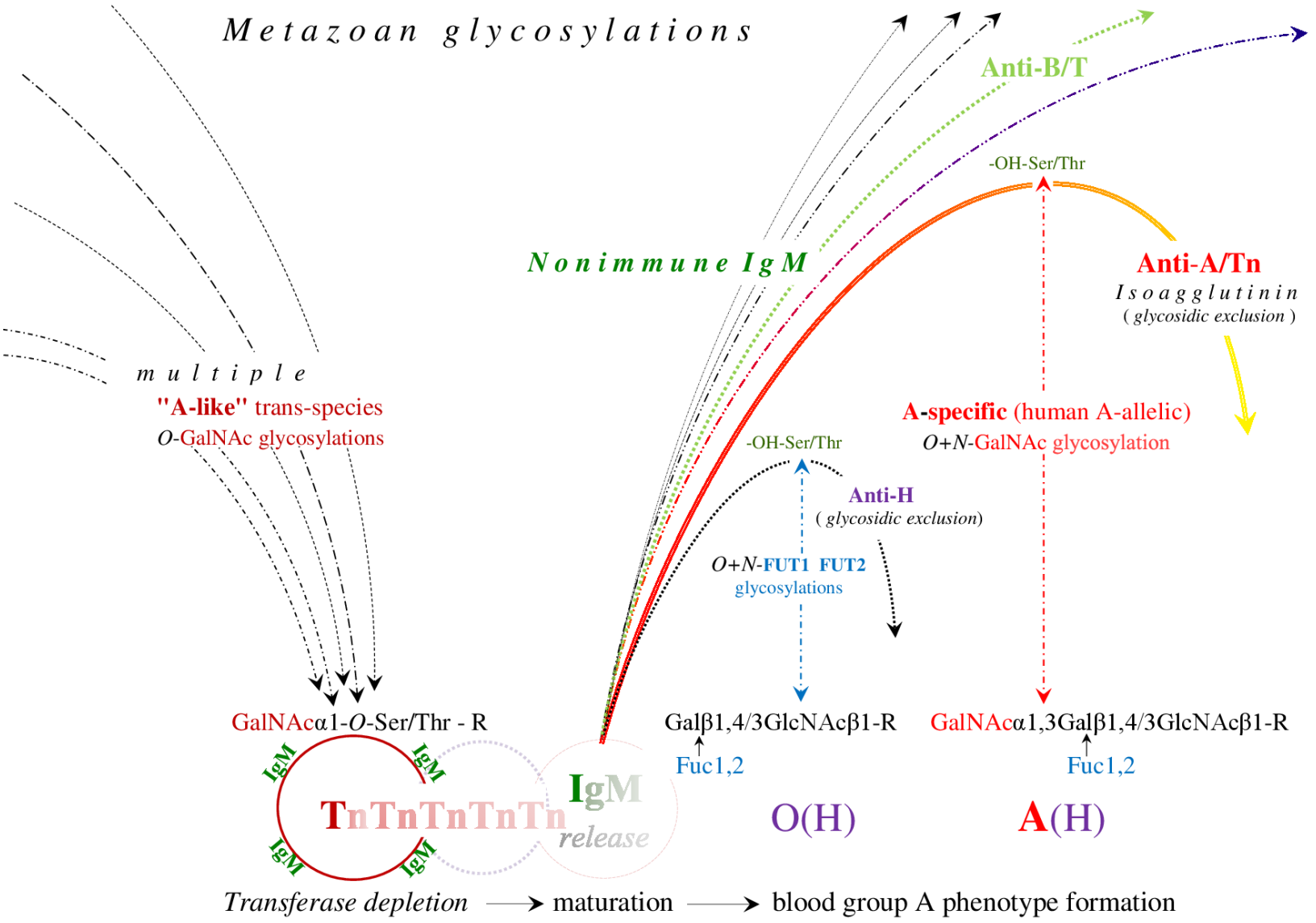

Figure 1

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Figure 1. The secretory polyreactive nonimmune IgM is an *O*-glycan-depleted antibody molecule, exerting serine and threonine residues and has emerged from its cell adhesion version, through which it acts as the complementary protein of the A-like/Tn antigen and undergoes ABO phenotype establishment, occurring on both cell surfaces and plasma proteins**.** This process is based on human-specific FUT1 and FUT2 fucosylations, precluding the induction of significant expression of the anti-H antibody, which is restricted to the rare *Bombay type* (O(h)) individual [42][43]. The plasma of O(H) blood group individuals exhibits strong anti-A/Tn reactive IgM or anti-A isoagglutinin activity, implicating secondary adaptive IgG production. In blood group A, the appearance of the ancestral anti-A activity is reduced or excluded by human-specific A-allelic GalNAc glycosylation, independent of classic clonal selection, hypothetically allowing the conversion of synthesized glycoconjugates into phenotype-specific plasma glycoproteins and/or molecular complexes that become subject to internalization [44]. The graphic (with minor changes) was constructed based on Fig. 4 in the manuscript by Arend (2017) [29].

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Figure 2

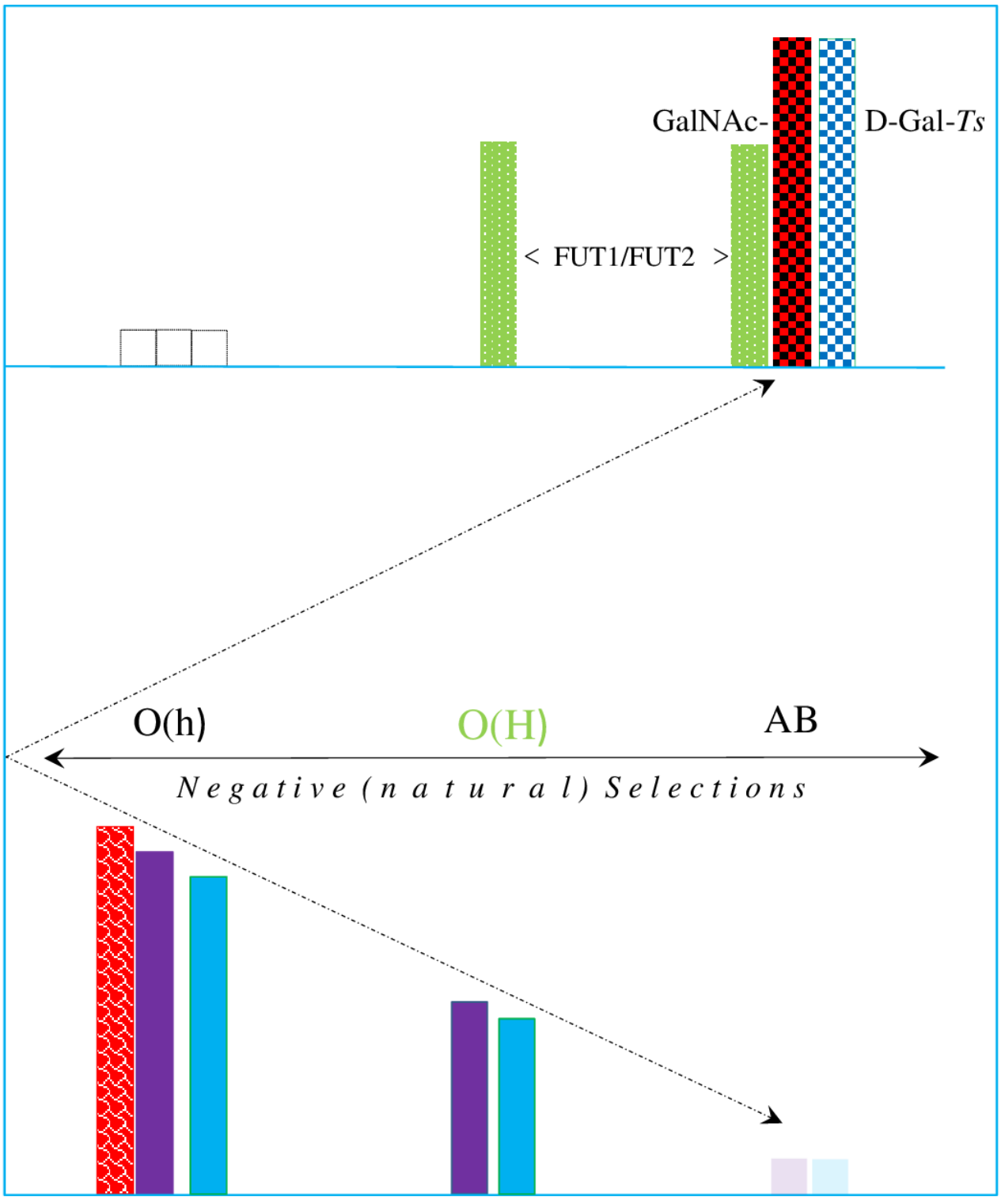

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Figure 2. Virtual relationships between the degree of ABO(H) phenotype-specific glycosylations and corresponding innate antibody levels. The non-phenotype O(h) or *Bombay type* and blood group AB appear to mark two opposite directions of negative (natural) selection and demonstrate how phenotype and isoagglutinin production form evolutionary functional unity, in which the degree of phenotype diversity and innate immunity are inversely proportional. Because statistical data are not available from the Bombay type due to its small population size, the isoagglutinin levels are estimated according to existing reports and the hypothetical serological profile of the classic *Bombay type* (h/h; se/se) [42], which is characterized by the complete lack of ABO blood group glycosylations.

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Why life-threatening malaria is especially prominent in non-O blood groups.

Brooks and Mclennan (1992) [45] argued that *P. falciparum*, transmitted to humans by *Anopheles gambiae*, has no alternative vertebrate reservoir hosts. We know that this parasite does not have the cellular machinery to complete protein glycosylation independently because it lacks the ability to synthesize the amino sugar GalNAc and to glycosate *O*-GalNAc, while intriguingly expressing abundant serine residues. Moreover, considering that malaria tropica infections occur regardless of the ABO blood group but life-threatening disease develops almost exclusively in non-O blood groups, the human glycoproteome must play a critical role in these infections. Indeed, these infections occur through two genetically distinct glycosylation pathways of the amino sugar GalNAc: 1) non-somatic, blood group-independent, serologically A-like or intermediate Tn-reactive *O*-GalNAc-glycosylation and 2) somatic glycosylation and formation of human mucin-type ABO-allelic structures or phenotypes.

As explained earlier, after the transmission of infectious *P. falciparum* sporozoites, their characteristic and abundantly synthesized serine residues and/or phosphorylated serine residues [13], arising throughout the parasite's life -cycle, access the human A-like intermediate Tn-reactive *O*-GalNAc-glycosylations. *P. falciparum* reaches every tissue, with the liver, bone marrow, and erythrocytic stages being the most critical stages during the life cycle progression. However when syngeneic glycosylation of peptides mainly occurs intracellularly, hybrid glycosylation or hybrid, mucin-type ABO blood group antigen formation may occur extracellularly, enabled by abundant, soluble glycotransferases and energy-rich monosaccharides released from activated platelets [41], which play a dominant role in malaria pathogenesis [46].

The complex invasion mechanism utilized by *P. falciparum* has been comprehensively explained in a recent study by Belachew (2018) [47]. In humans, this invasion implicates a subsequent molecular step for the access of the parasite to the mucin-type version of the ABO allele-specific phenotype

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formation, which occurs via both intracellular and extracellular *O*-linked glycosylation in epithelial and some endothelial tissues. It is noteworthy that expression of A- and B-transferring glycotransferases is independent of the secretor status [48][49] and that the mucin-type expressions of A, B and (OH) mucin-type glycans are exclusively dependent on the A, B and O(H) genotype [50], regardless of the Lewis type [51]. Glycophorins, which are the intrinsic components of the RBC membrane, provide abundant *O*-linked [52] [53] blood group A-, B- and Tn-reactive [54] oligosaccharides and appear to be identified as pathogen receptors and merozoite ligands of *P. falciparum* [55]. These hypothetical interactions are consistent with earlier observations by Barragan et al. (2000) [56], suggesting that soluble blood group antigens can inhibit the binding between the parasite and human RBCs. Appropriately enough, blood group A and B trisaccharide haptens inhibit parasite adhesion to the RBC surface, although notably stronger inhibition can be achieved with the synthetic disaccharide Galβ1-3GalNAcα1-O-Ser/Thr or T antigen coupled to bovine serum albumin [57]. Intriguingly the main rosetting ligand and recognition protein of the parasite, called *P. falciparum* erythrocyte membrane protein 1 (*Pf*EMP1), appears to even discriminate between blood group A qualities or subgroups [58] and thus demonstrates the role of this protein for infection and blood group-dependent severity of malaria disease.

The establishment of ABO mucin-type phenotypes occurs via identical glycosylation of both the cell surface and plasma proteins, including nonimmune IgM [28][29]. This *O*-glycan-depleted molecule hypothetically undergoes phenotypic accommodation through phenotype-specific *O*-glycosylation of serine/threonine residues at the V or Fc immunoglobulin regions [22][23]. Furthermore, ABO(H) mucin-type glycoprotein formation, arising during *P. falciparum* infection, suggests that hybrid *O*-glycan bridges are formed between parasitic serine residues and IgM [59], which becomes the basis of erythrocyte rosette formation. According to Rowe et al. (2002) [60], nonimmune IgM but not IgG binds to the surface of *Plasmodium falciparum*-infected erythrocytes owing to the

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number of acceptor sites (Figs. 3, 4); however, other observations suggest that only additional cross-linking functions of functionally synergistic α2-macroglobulin makes this adhesion possible [61]. While the details of the involvement of these macromolecules in rosette formation are thus still unknown, this hypothetically occurs according to the principle of glycosidic exclusion or phenotypic accommodation of plasma proteins, as published by this author [28][29]: in the case of malaria infection, the phenotype-determining glycotransferases catalyze the binding of IgM and alpha-2 macroglobulin to both infected erythrocyte surfaces as well as the serine-rich peptides of the parasite.

Apart from these still open questions on the binding mechanism, recent observations show the strongly protective role of the IgM molecule in *P. falciparum* infections regardless of blood group phenotype: in a comprehensive investigation, Boyle et al. (2019) demonstrate how naturally acquired IgM blocks merozoite invasion of red blood cells in a complement-dependent manner, whereas high IgM levels in conjunction with IgG are associated with a significantly reduced risk of clinical malaria in a longitudinal cohort of children [62].

The role of fucosylation in *P. falciparum* infections remains elusive; *O*-fucosylation performed by the parasite was recently described [63] and the involvement of host-provided hybrid fucosylations has been suggested by this author in a recent review [1]. Human-specific α1,2 L-fucosylation (FUT1/FUT2) represents the basis of ABO-blood-group phenotype formation on the cell surfaces and plasma proteins, and the O(H) type appears to stand for controlled or physiological self-reactivity [29][43], regulating the expression of intermediate A-like/Tn epitope via dynamic activities of the nonimmune IgM or anti-A/Tn isoagglutinin.

Although this concept, in which cell surface enzymes and plasma proteins act together synergistically awaits experimental confirmation, a comprehensive study by Carlson et al. as early as 1992 [64] strongly supported this concept: Rosettes of a blood group A-preferring parasite could be disrupted only when grown in blood group O or B RBC cultures and the rosettes of a blood group B-

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preferring parasite could be more easily disrupted when grown in blood group O or A RBC cultures. This phenomenon is explainable only by the activity of the corresponding residual anti-A and anti-B isoagglutinin, still adhering to the culture RBCs. Furthermore, in the same study rosetting of parasites, grown in blood group A or B RBC cultures, was specifically inhibited by the terminal mono- and trisaccharides of the A and the B blood group antigens, whereas fucose and H-disaccharide (D-gal[1-3]a-1, fuc) appropriately inhibit rosette formation in all ABO blood groups to some extent. In the present study rosette formations by O(H) RBCs occurred identically in one individual with the O(h) *Bombay* type, which might reconfirm the phenotype-independent infection of *P. falciparum*, meaning binding between parasite and host via trans-species A-like/Tn formation. This does not preclude the additional binding of the parasite to O(H) RBCs by fucosylation (Fig. 4).

Furthermore, ABO mucin-type generation [65] involves fucosylation (Fig. 3), which protects from autoreactive anti-H activity during phenotypic accommodation of plasma proteins [28]. This mechanism may also enable the hybrid fucosylation between the parasite and blood group O(H) RBCs, which thus have the least contact with the parasite when compared with those of non-O blood groups (Fig. 3). Moreover, without A and B blood group-determining glycotransferases, O(H) can maintain nonimmune IgM and adaptive secondary IgG against syngeneic and hybrid A and B antigens, making it the most protected blood group (Fig. 3). This special immunological position of the O(H) phenotype was hinted at in an early study [66]. It has been discussed recently [1] and again becomes evident: innate IgM, which physiologically regulates the expression of the syngeneic intermediate A-like/Tn antigen in blood group O(H) [28][29], will attack the formation of hybrid A and B formations, which are based on foreign (parasitic) peptides, and the quality and extent of this interaction should become a topic in future experiments. Blood type A phenotype formation merely maintains nonimmune anti-B-reactive IgM and blood type B maintains nonimmune anti-A/Tn reactivity that might protect against hybrid Tn formation but does not affect the formation of T and B

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cross-reactive hybrid glycoproteins. The blood group AB enables the strongest contact with the pathogen, and molecularly precluding any isoagglutinin activity, makes this group the least protected and smallest among the ABO-blood groups (Fig. 3).

Although the formation of hybrid carbohydrates is accomplished by circumventing innate immunity, the adhesion of these structures to parasite proteins ultimately makes non-O blood groups an immunological target. The physiological lack of innate anti-A and anti-B antibodies poses the following immunological dilemma in these blood groups: on one side, it protects these blood groups from self-reactivity, but on the other side, it cannot prevent the formation of autoantigenic targets in subsequent pathogenic steps during malaria infection. This is evident in recent observations [67][68], wherein the induction of autoimmune processes contributes to the development of severe malaria disease, most likely even dominated by autoimmune inflammations.

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Figure 3

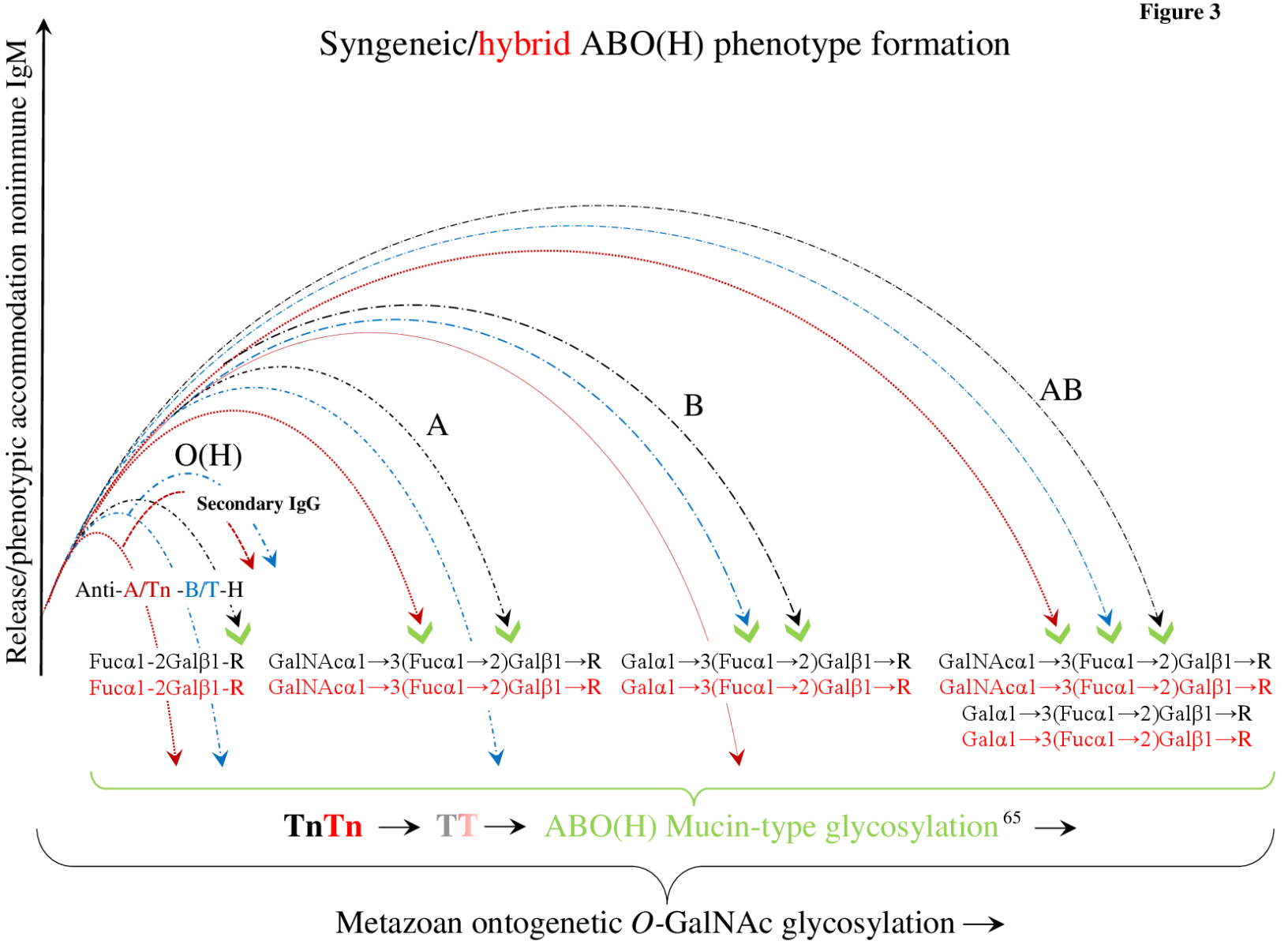

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Figure 3. ABO blood group phenotype development occurs on both cell surfaces and plasma proteins and ABO-associated mucin-type glycoprotein development on epithelial cell surfaces and plasma proteins arises with carbohydrate chain elongation. This elongation is controlled by the synthesis of Galβ1-3GalNAcα1-O-Ser/Thr or T antigen [30] and is completed through ABO allelic *O*-glycosylation. During *P. falciparum* infection, ABO phenotype development generates hybrid antigenic structures. In the O(H) blood group, glycosidic accommodation neutralizes innate anti-H reactivity. In these individuals, the parasite can adhere to RBCs via mucin-type fucosylation, which does not affect anti-A/Tn and B/T-reactivity of germline-encoded nonimmune IgM. This nonimmune antibody, which regulates the expression of the syngeneic intermediate A-like/Tn antigen, interacts with the formation of hybrid A and B formations to an unknown extent in association with a secondary IgG response. Non-O blood groups neutralize IgM's anti-A/Tn and B/T reactivity, while clonal selection prevents adaptive IgG response. Thus, O(H)-blood group individuals exhibit both innate and adaptive immunity against syngeneic and hybrid A/Tn- and B/T antigens. In contrast, A blood group individuals exclusively maintain their innate anti-B/T cross-reactivity against syngeneic and hybrid B/T antigen, while B blood group individuals maintain innate anti-A/Tn against syngeneic and hybrid A/Tn antigen, which does not affect the formation of T and B cross-reactive hybrid glycans. Finally, AB individuals exhibit the strongest contact with *P. falciparum*, with their phenotype precluding any corresponding antibody response.

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Figure 4

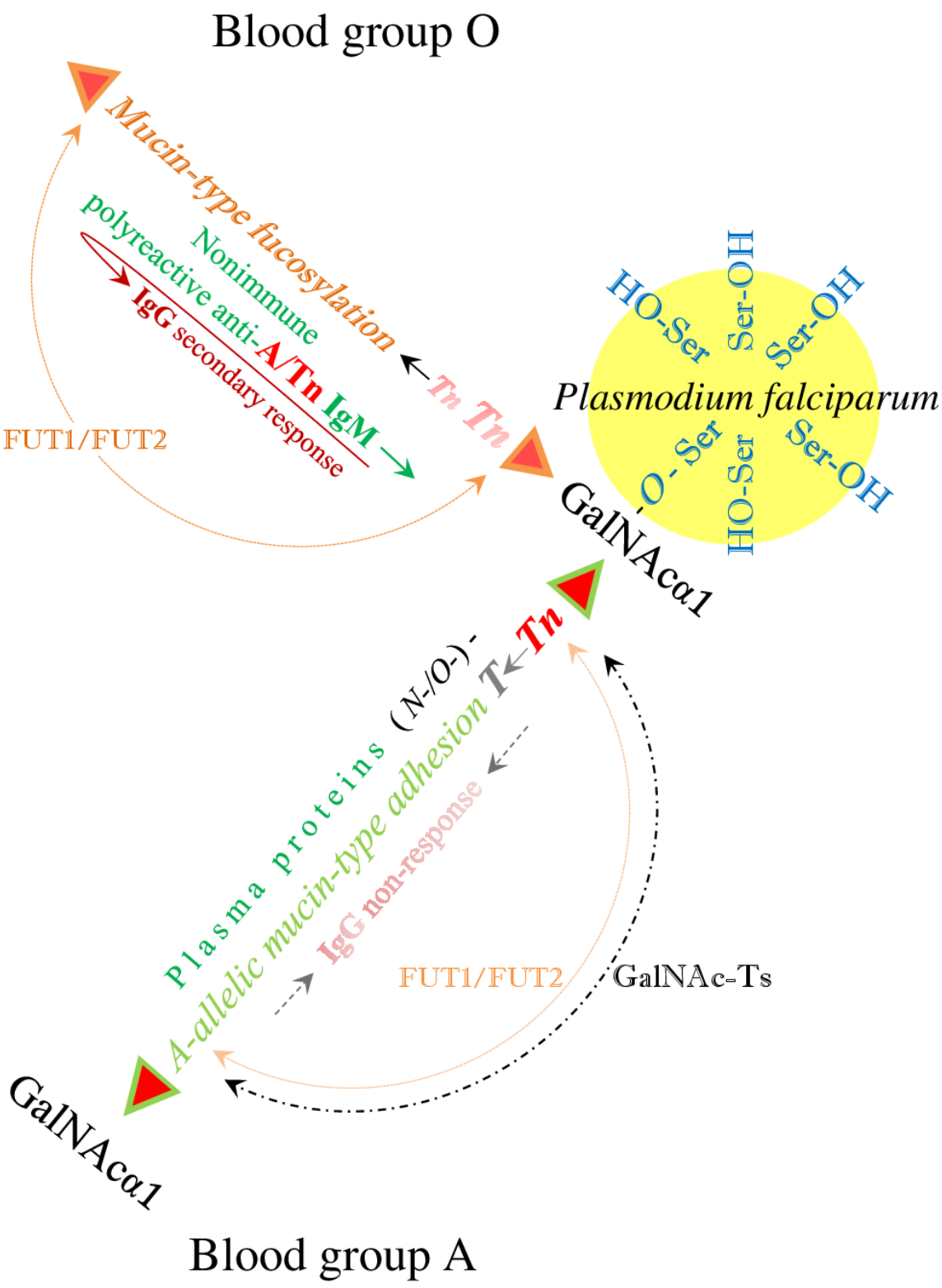

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Figure 4. The adhesion of the parasite to O(H) RBCs is initiated blood group-independently via fleeting hybrid A-like/Tn formation and is completed by mucin-type fucosylation. This process does not affect anti-A/Tn reactivity of nonimmune IgM, which is implicated in the control of syngeneic and the strong interaction with hybrid A-like/Tn expression. Accordingly, pathogen adhesion to A blood group RBCs occurs through fucosylation-driven A-allelic mucin-type formation. This process precludes adaptive and innate antibody reactivity against both syngeneic and hybrid A-like/Tn antigens due to clonal selection and phenotype-associated plasma glycosylation. In these conditions, nonimmune IgM is an adhesion molecule. This figure expands on Fig. 2 of a previous publication [1].

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Conclusions

My previous paper proposed self-destructive biological altruism of the host [1], which enables the survival of the malaria parasite *P. falciparum*. This phenomenon has now been identified as a defined molecular biological or biochemical event, involving two genetically different GalNAc glycosylation steps: in the first step pathogen transmission occurs regardless of the blood group across species barriers via hybrid syntheses or molecular mimicry and shows another evolutionary function of Tn. The chemical simplicity of this antigen does not stand for antigenic and functional unity [69], whereas during malaria infection, the undefined biophysical properties of the GalNAc molecule access (via *O*-glycosidic linkage) the complex metabolism of *P. falciparum's* amino acid serine. The prominent evolutionary role of this amino acid becomes evident also in other host-pathogen interactions, such as *Entamoeba histolytica* infection, in which the serine-rich *E. histolytica* protein [70][71] dictates the binding and virulence of the parasite [72]. Thus, the proposed concept of malaria tropica infection might reveal a principle of parasite - host coevolution, which is initiated by trans-species, hybrid Tn antigen formation. This genetically undefined serologically A-like structure must always be differentiated from the human blood group A-specific epitope, which is encoded by the A allele of the ABO gene located on chromosome 9q34 and which together with the B-allele determines the risk of developing life-threatening disease in the non-O blood groups A, B and AB in a second glycosidic, genetically defined step.

The predominant occurrence of life-threatening disease in individuals with non-O individuals strongly supports the above argument by Brooks and McLennan (1992) [45] that survival of *P. falciparum* depends on the infection of the human host, although it is important to note that according to a new coalescence analysis by Yamamoto et al. (2014) [73], blood group A was again confirmed as the primordial blood group structure, which appeared first during evolution. Meanwhile, existing research suggests that over millions of years, this primordial structure has undergone the

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selective pressure of evolutionarily selective diseases such as malaria to yield the current global distribution of ABO phenotypes. Although the potential impact of human - *P. falciparum* coevolution on the development of the ABO(H) blood groups and human immune system was comprehensively discussed by Cserti-Gazdewich [74], patterns of O(H) blood group occurrence are consistent with natural selection in response to *P. falciparum* infection, which favors the survival of O(H) individuals in malaria-endemic regions [75]. Although recent observations additionally account for a survival advantage of secretors over non-secretors [76] and participation of the Se and Lewis genes in malaria pathogenesis, the fundament of malaria tropica infection and disease in humans remains the inability of the parasite to synthesize the amino sugar GalNAc and to perform A-like *O*-GalNAcα1-Ser/Thr-R or Tn formation. Furthermore, the subsequent somatic pathway of the GalNAc molecule, together with the sugars galactose and fucose, determines the evolutionary functional unity of ABO phenotype development with corresponding immunoglobulin production and explain the biological conditions for malaria infections, as well as the disease trajectories between O(H) and non-O phenotypes.

Cancer mortality may statistically interfere with malaria rates to some extent. In recent retrospective studies, performed on two different Asian populations, cancer mortality was highest in blood group A [77][78]. It is not surprising that non-O blood groups are the ideal target of *P. falciparum* owing to comprehensive presentation of both A-like/Tn and A/B allelic glycosylations, whereas the most ideal target should be the early cancer cell, generating a surplus of genetically undefined A-like *O*-GalNAc glycans, which might explain the above cited, recently observed inverse relationship between global malaria incidence and cancer mortality [17]. According to this report, cancer mortality was significantly reduced in malaria-endemic regions. This phenomenon awaits validation by studying the survival patterns of human tumors transplanted into non-primate animals with insufficient innate immunity or severe combined immunodeficiency, for example the

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castaneous mouse (CAST) [79] or SCID mouse [80], infected with *P. falciparum*. The insight into the interaction mechanisms of this parasite with cancer metabolism might provoke speculations on completely new, future therapeutic strategies for cancer. Intriguingly, a glycosaminoglycan binding malaria protein, targeting human cancer has been discovered by Salanti et al. [81]

Although *P. falciparum* cannot survive in non-primate animals, most likely resulting from innate immunity, in humans, infections occur regardless of the blood group. *P. falciparum* parasitemia does not differ between non-O and O(H) blood groups in malaria-endemic areas [82], life-threatening infections, however, are more frequently diagnosed in non-O(H) phenotypes [83][84], wherein the number of erythrocyte rosette formations reflects the severity of the disease [85][86]. Clearly, blood group phenotypes determine innate immunity: over the decades several concepts have been developed, in which the pathogen evades host immunity, and depending on the experimental approach and/or the interpretation of existing data, different cellular and molecular aspects of blood group A-favored infection become evident: in a model by Moll et al (2015) [87], the patient's RBC surrounds itself with normal RBC, which makes the pathogen's antigen inaccessible to yet undefined antibody activity and clearance by the immune system, whereas in my concept the pathogen utilizes the physiological absence of the corresponding antibody or phenotype-specific isoagglutinin, which has been neutralized by A, B or AB phenotypic glycosylation. ABO epitopes and innate isoagglutinin activities arise molecularly together during the same enzymatic process and represent a developmental and evolutionary unity. The overexpression of isoagglutinins in the *Bombay* type and their complete downregulation in blood group AB mark the opposite ends of the spectrum of ABO phenotype diversity in natural selection, whereas the central immunological position of the human blood group O(H) guarantees the maintenance of species and phenotype diversity via a mechanism of immunologically-controlled anti-self-reactivity during growth and reproduction [42]. Consequently, the blood group AB is the least protected and

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remains the smallest among the ABO blood groups. In contrast, O(H) individuals have the least molecular contact with the pathogen, rarely develop severe disease, and survive this coevolution in an immunological balance with the pathogen as the largest blood group worldwide. Although the significance of host - parasite relationships such as host identification of foreignness, host susceptibility to parasites, and antibody responses have been discussed for decades as causes of the present-day world distribution of human ABO blood groups [88][89], this distribution may be explained through the coevolution of humans and *P. falciparum* [74] on a molecular and immunological basis and demonstrate how augmenting phenotype diversity is associated with decreasing immunity.

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