ABO phenotype-protected reproduction based on human specific α1, 2 L-fucosylation as explained by the *Bombay* type formation

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Abstract.

The metabolic relationship between the formation of the ABO(H) blood group phenotype and human fertility is evident in the case of the (Oh) or Bombay blood type, which Charles Darwin would have interpreted as resulting from reduced male fertility in consanguinities, based on the history of his own family, the Darwin/Wedgewood Dynasty. The classic Bombay type occurs with the extremely rare, human-specific genotype (h/h; se/se), which (due to point mutations) does not encode fucosyltransferases 1(FUT1) and 2 (FUT2). These enzymes are the basis for ABO(H) phenotype formation on the cell surfaces and fucosylation of plasma proteins, involving neonatal immunoglobulin M (IgM). In the normal human blood group O(H), which is not protected by clonal selection with regard to environmental A/B immunization, the plasma contains a mixture of non-immune and adaptive anti-A/B reactive isoagglutinins, which in the O(h) *Bombay* type show extremely elevated levels, associated with decreased levels of fucosylation-dependent functional plasma proteins, suchs as the van Willebrand factor (vWF) and clotting factor VIII. In fact, while the involvement of adaptive immunoglobulins remains unknown, poor fucosylation may explain the polyreactivity in the *Bombay* type plasma, which exhibits pronounced complement-binding cross-reactive anti-A/Tn and anti-B IgM levels, with additional anti-H reactivity, acting over a wide range of temperatures, with an amplitude at 37 °C. This aggressive anti-glycan-reactive IgM molecule suggests the induction of ADCC (antibody-dependent) and/or complement-mediated cytotoxicity via overexpressed glycosidic bond sites against the embryogenic stem cell-to-germ cell transformation, which is characterized by fleeting appearances of A-like, developmental trans-species GalNAcα1-*O*-Ser/Thr-R glycan, also referred to as the Tn (T “nouvelle”) antigen.

Keywords: ABO-phenotype-protected reproduction, glycosidic exclusion, glycosidic accommodation, somatic fucosylation, AB antigen completeness, inbreeding depression, epistatic genetic interaction.

Abbreviations: ADCC = antibody-dependent (mediated) cellular cytotoxicity. A2M= α2-macroglobulin; Ab = antibody; EGF= epidermal growth factor; Ig = immunoglobulin; IgG = immunoglobulin G; IgM = immunoglobulin M; GC = germ cell(s); ESC = embryonic stem cell(s); D-GalNAc = *N*-acetyl-D-galactosamine; D-Gal = D-galactose; FUT1 and FUT2 = galactoside 2-alpha-L-fucosyltransferase 1 and 2; vWF = van Willebrand factor.

Introduction

The potentially protective role of the A-allele in human fertility and the position of its encoded GalNAc transferase (Arend, 2012; 2014; 2016) have recently become topics of active controversy. The initial reports on lower counts of ovulation-competent eggs in blood group O females (Nejat et al., 2011) have not been confirmed (Spitzer et al., 2014), and the pronounced ovarian hyperstimulation in females with blood group A (Binder et al., 2008) could not be documented in subsequent studies (Pereira et al., 2015). Similarly, the prevalence of male infertility in blood group O males (Khan et al., 2010) was not confirmed in a more recent study (Prasad et al., 2015). These conflicting results may reflect the confounding effect of hidden ethnic diversity in the analysis of genetic statistics from any given contemporary population, in addition to issues with non-comparable experimental designs and techniques. Moreover, the classic definition of the ABO(H) phenotype has recently been questioned; in particular, analyses of the AO exon and intron in individuals with an "A- weak B" phenotype have revealed a novel O1v-A2 hybrid allele that results in missense mutations in the A-transferases and/or transferees (Hosseini-Maaf et al., 2005). Additionally, unusual O alleles (Yazer et al. 2008), including O2 have been described at the ABO locus, which have also been implicated in the unexpected blood group phenotypes. Thus, the human blood group O(H) can no longer be considered a genetic entity (Arend, 2016). Finally, this ongoing debate has largely neglected the established epistatic cooperation between the functions of the A and B alleles located on chromosome 9, and the functions of the H and Se loci on chromosome 19 at 19q13.3, which encode the fucosyltransferases 1 (FUT1) and FUT2 that ultimately promote the synthesis of the ABO(H) phenotypes on red cells and plasma proteins such as vWF and clotting factor VIII (O'Sullivan et al., 2016) carried by A2M (Matsui et al., 1993). Thus, the ABO(H) phenotype formation occurs on both, the cell surfaces and plasma proteins, and blood group A/B/H-determining functional glycotransferases act in every plasma independently of the secretor status (Sawicka 1971; Schenkel-Brunner 1995), which was recently confirmed by conversion studies (Hult et al. 2017). Consequently, the secretor-independent ABO(H) reactivities of A2M, vWF and factor VIII are strictly expressed according to the phenotype (Matsui et al., 1993; 2001). A2M is considered an evolutionarily conserved arm of the innate immune system (Armstrong and Quigley 1999) and works synergistically with the structurally related, ancestral non-immune IgM molecule (Stevenson et al., 2015). These molecular biological connections are demonstrated by the

opposite phenomenon observed in Indian families and in the extremely rare and "real" blood group O, also referred to as Oh or *Bombay* type (Bhende et al., 2008), found in 1 of 10,000 individuals in India and 1 in a million people in Europe (Balgir, 2005).

Bombay type formation as it relates to the lack of α1,2 L-fucosylation

The postulated cooperation between enzymatic interactions on the cell surface and plasma proteins in the construction of the ABO(H) histo-blood group phenotype (Arend 2016; Arend 2017) may best be explained by considering the rare Oh or *Bombay* blood type (h/h; se/se), which is defined by the native structure Gal-β1-R. The functionality of this structure has been shown in conversion studies *in vitro* (Schenkel-Brunner et al., 1975; Beyer et al., 1980), and may mimic the starting point or end of the evolutionary enzyme cascade, resulting in the formation of the ABO(H) phenotype **(Fig. 1)**. This Indian Oh or H-null *Bombay* type must be differentiated from the H-weak blood groups discovered on Reunion Island (Fernandez-Mateos et al., 1998) and the various *Para-Bombay* types, which are determined by the genetically distinct α-L-fucosyltransferase FUT2-encoding Se gene. Because the differential diagnosis of these blood types in clinical practice is difficult, these blood types are also regarded as "blind spots" in transfusion medicine (Chacko et al., 2011), and they are differentiated based on the formation of Lewis groups and the H-antigen/receptor, which in *Para-Bombay* types is typically expressed in mucoepithelial cells and in the secretions of ABO(H) blood group secretors (Watkins, 1980; Wang et al., 1997), as recently reviewed by Dean (2005). In the native *Bombay* type, based on the extremely rare genotype (h/h; se/se), the FUT1 and FUT2 transferases are not encoded and none of the ABO(H) epitopes are synthesized. Moreover the ABO(H) reactivity of the plasma proteins vWF and clotting factor VIII is markedly decreased (O'Donnell et al., 2005), reflecting the identical non-fucosylation of plasma proteins, while the levels of the complement-binding anti-A/ Tn-cross-reactive and anti-B reactive IgM or isoagglutinins are markedly increased and exert an additional strong anti-H-reactivity, acting over a wide range of temperatures, with an amplitude at 37^{o}C. The *Bombay* type organism is not protected by clonal selection, with respect to environmental immunisation by A/B cross-reactive structures, and the anti-A/B and anti-H plasma levels thus reflect a mixture of innate (germline-encoded) and adaptive reactivities. As suggested by the other, above mentioned functional plasma proteins, these increased isoagglutinin and unusual anti-H reactivities may be caused by non-fucosylation, involving further glycan depletions, while the involvement of adaptive immunoglobulins remains

unknown. Because antibodies, produced for therapeutic applications (Yamane-Ohnuki & Satoh, 2009; Liu, 2015), have shown that the potency of the immunoglobulin G molecule in initiating ADCC can be increased 50-fold simply by removing the single fucosyl residue from the Fc glycan (Shade & Anthony, 2013), and the seminal plasma of infertile leukospermic men has been reported to exhibit high levels of poorly fucosylated IgG (Kratz et al., 2014), it could be assumed that the unusual anti-H and anti-A/B reactivities of the *Bombay* type plasma initiates ADCC, promoting an anti-self-reactive inflammatory process that affects male gamete performances. However, according to Rumpold et al. (1981) and Perlmann et al. (1985), ADCC may not to be mediated by IgM alone, and traces of IgG are always needed; ddditionally, the 1,2 defucosylation of IgG, in particular, has not been reported to initiate ADCC. However, these studies were performed on artificially constructed (non-native) immune antibodies and IgG subclasses that were defucosylated *in vitro*. Indeed, due to the small population size, there are no established studies so far on the naturally non-fucosylated, glycan-depleted proteins in *Bombay* type plasma, in which the aggressive germline-encoded IgM molecule potentially cooperates with IgG.

In the normal human blood group O(H), which represents the most common blood group worldwide, non-immune IgM reactivity is associated with adaptive, internal and environmental induction of IgG, and appears to be involved in regulating the developmental Tn (Moreau et al. 1957) and T formation (Friedenreich & Munck, 1930) in germ cell production and cell renewal (Arend, 2017). This function may contribute to the potential survival advantage of the blood group O(H) individual, related to the overall risk of developing cancer (Zhang et al., 2014; Hsiao et al., 2015). The evolution of the blood group O(H) may thus provide an immunological balance between regulatory, anti-self-reactive and aberrant glycosylation processes **(Fig. 2).** In the blood group A, in which adaptive anti-A/B cross-reactive IgG formation does not occur due to clonal selection, the phenotypic glycosidic accommodation of plasma proteins has reduced or removed such reactivities from the polyreactive non-immune IgM and may contribute to the statistically increased susceptibility of this groups to distinct types of cancer. The extreme opposite of both blood group O(H) and blood group A formation occurs in the *Bombay* type individual, in whom the total lack of somatic, ABO(H)-phenotypic glycosylation maintains the natural polyreactivity and autoreactivity of this molecule. Independently of the phenotype, these ancestral reactivities arise from non-somatic, developmental, trans-species glycosylation-deglycosylation processes, in which GalNAc1α-*O*-Ser/Thr formation characterizes ESC fidelity

(Reisner et al., 1978; Nash et al., 2007) in metazoan GC transformation and/or cell renewal (Arend, 2017), while undefined ancestral fucosylated glycoconjugates (Alonso et al. 2003; Fazel et al. 1990) and primate-intrinsic fucosylations (Dupuy et al. 2002; Rabionet et al. 2008) potentially arise with the enzymatic cascade of complex developmental *N*/*O* glycan formations, in which the timing between fucosylations and GalNac-glycosylations remains unknown. However, the aggressive anti-H reactivity in the *Bombay*-non-phenotype, which primarily reflects the lack of α1,2 fucosylation, may indicate that in normal conditions ontogenic fucosylation precedes GalNAc glycosylations. Thus, in poorly fucosylated, glycan-depleted *Bombay* type plasma, the polyreactive IgM highly suggests (via overexpression of augmented glycosidic sites) the induction of a complex, ADCC and/or complement-mediated cytotoxicity against the embryogenic stem cell to germ cell transformation, dominated by serologically A-like, developmental trans-species GalNAcα1-*O*-Ser/Thr-R glycan formations. These suggestions await experimental confirmations, while the relationsship between the *Bombay* non-phenotype development and reduced fertility with the overexpression of adaptive and innate immunity is evident.

The role of fucosylation in complex immunoglobulin functions

The complex mechanism underlying immunoglobulin fucosylation and its significance in growth and development have been a principal focus of molecular biological research for decades. As early as 1973, Andersson & Melchers reported that the polymerization of intracellular IgM to the 19 S unit occurs during the process of secretion, whereas galactose and fucose are added to the molecule immediately prior to secretion. Moreover, Kyogashima et al. (1989) described that the presence of fucosyl residues in a certain sugar sequence may signify the early stages of tumor growth and could increase the susceptibility of the tumor cell to antibody-mediated cytotoxicity when fucosyl residues accumulate on the tumor cell surface. The core fucosylation of the μ-heavy chains regulates the assembly and intracellular signaling of precursor B cell receptors (Lin et al., 2014), and studies have established that low fucose levels predominantly enhance natural killer- or mononuclear cell-mediated ADCC, whereas polymorphonuclear cells have been described to preferentially kill cells via a highly fucosylated antibody (Peipp et al., 2008). Several recent reviews have focused on the growing knowledge regarding the different functions of the fucose molecule in *N*- and *O*-linked glycans (Gabius et al., 2011; Zhao et al., 2012), and there is growing interest in the significance of fucosylation in fertility and reproduction in both

prokaryotes and eukaryotes (Ma et al., 2006; Gabius, 2009). In translational studies, a mammalian RNA incorporated into *Xenopus laevis* oocytes initiated the synthesis of mammalian immunoglobulin in connection with L-fucosylation (Deacon and Ebringer, 1977), and additionally, cellular fucosylation events in FX(-/-) mice were identified as essential components of fertility, early growth, and development (Smith et al. 2002). The obviously positive impact of fucosylation on the metabolic and/or immunological agreement between self and non-self may be visible in ABO(H)-incompatible tissue transplantation. Recent studies in humans have shown that the FUT VI transferase-enforced fucosylation of cord blood hematopoietic cells led to improved engraftment of neutrophils and platelets (Popat et al., 2015). In addition, these studies on human multipotent mesenchymal stromal cells (Sackstein et al., 2008), along with the efficient *ex vivo* fucosylation by FUT VI and FUT VII transferases in murine models (Taupin, 2010; Robinson et al., 2014) have suggested that the protective effect of the fucose molecule on tissue transplantation may also include the reduction of early infections. However, such external fucosylation may not substitute the physiological developmental functions of the human-specific FUT1- and FUT2-mediated fucosylation, which ultimately enables the complex formation of the A/B-phenotype. Currently, the functions of these enzymes and their epistatic connectivity cannot be substituted by therapeutic equivalents, and the ultimate "teaching experiment" i.e. the transplantation of an A/B graft to a FUT1- and FUT2-depleted O(h) *Bombay* type organism, might not be feasible.

Concomitant formation of the ABO(H) phenotype and isoagglutinin specificities is based on α1,2-L-fucosylation

In contrast to the environmentally-induced formation of naturally-occurring antibodies, which is accomplished by mature B cells as a response to microbial lipopolysaccharides, and controlled by the classic clonal selection, the innate or ancestral trans-species reactive IgM in vertebrates (Longworth et al., 1973; Czajkowsky et al., 2010; Buchman, 2014) is not exclusively produced by B cells and was likely present at the inception of the adaptive immune system (Rumfelt et al., 2001). In addition, exogenous immunizations of the C57BL/10 strain of mice by lipopolysaccharides from *E.coli*:086 has been shown to induce the formation of strong anti-A/B cross-reactive antibodies but did not increase the preexisting levels of innate anti-A-reactive IgM (Arend 1971). Moreover, neither *N*- nor *O*-linked native blood group A-like glycans have yet been demonstrated in prokaryotic microorganisms. For instance, the serologically A-like, mucin-type GalNAc-*O*-

Ser/Thr glycosylation does not occur in bacteria (Bennett et al., 2012), whereas the *O*-linked GalNAc glycan-bearing ovarian glycolipids, discovered in the C57BL/10 strain of mice (Arend and Nijssen, 1976), are associated with the formation of a syngeneic, complementary anti-A/Tn cross-reactive immunoglobulin M (IgM), which demonstrates identical serological reaction patterns to the human innate anti-A isoagglutinin and does not appear in animals that have undergone an ovariectomy prior to the onset of puberty (Arend, P. and Nijssen, 1977; 1977a). The ancestral, innate IgM molecule, which is produced as a non-immune glycoprotein during germ cell (GC) development and various other non-somatic developmental events, not only exhibits distinct, preformed antibody specificities but also exhibits a broad polyreactive complementarity, harboring the immunological history of the genome and/or species, and involves multiple self-incompatibilities. Consequently, maturing tissues in the developing metazoan organism are physiologically exposed to poly- and autoreactive IgM, which however in humans interacts with the glycotransferase-determined processes of phenotype formation via its multiple glycosidic sites. A previous report (Spalter et al., 1999) demonstrated that any normal human serum containing natural antibodies will react with autologous ABO blood group antigens in the micromolar range, and the authors suggested that the tolerance to autologous ABO blood group antigens may result from complementary interactions between the variable regions of the antibodies. However, it is striking that an ABO(H) epitope, which is missing on the red cell surface, is always reflected in a specific complementary domain on the surrounding secretory non-immune IgM molecules or isoagglutinins, which cannot be explained by clonal selection and provokes questions about how this "gap" between two opposite complementary sites is filled using a complementary glycan. Thus, according to the concept of glycosidic exclusion and accommodation, the development of ABO(H) phenotypes and mercaptoethanol-sensitive, preferably at 24^{o}C ABO(H)-reactive innate IgM or isoagglutinin specificities, which cannot be blocked by pharmaceutical immunosuppression (Chuang et al., 2008), occurs via identical enzymatic steps in a molecularly connected synthetic pathway (Arend, 2016). The impact of glycosylation on the biological function and specificities of immunoglobulins has been discussed for decades (Arnold et al., 2007; Cunneen et al., 2013). When it is independent of the classic clonal selection of adaptive immunoglobulins, that a glycosylation accomplishes "antibody clearance", (Huang et al., 2006), and α-GalNAc residues have been installed at the desired positions to protect (in a novel concept termed "carbohydrate as a protective group") some hydroxyl groups of threonine/serine residues (Yoshimura et al., 2010), the simultaneous appearance of an ABO(H) phenotype and its corre-

sponding innate (auto) antibody may be reduced or excluded by identical glycosylation of complementary domains on the cell surface and plasma proteins, as shown in Figure 1. In normal persons, this process is expected to be linked to the primarily polyreactive and autoreactive neonatal IgM, which appears to provide *N*- and *O*-glycosidic linkage options on the heavy and light chains (Arnold et al., 2005) that are involved in the dynamic placement of fucosyl residues added in connection with galactose (Andersson and Melchers, 1973) in a subsequent glycosylation. Studies have established that antibody-antigen reactions are reversible, since these reactions predominantly consist of non-covalent bonds, such as electrostatic interactions and hydrogen bonds, *van der Waals forces*, and hydrophobic interactions (Goldberg, 1952; Janeway et al., 2001). The enzymes involved in these reactions catalyse reactions in both directions, and the binding between carbohydrates and proteins does not represent a lock-and-key mechanism. Researchers have postulated that these dynamic fluctuations of the protein-carbohydrate interactions promote protein aggregation (Voynov et al., 2009), whereas the disruption of such interactions in the glycosylated domain of antibodies leads to the exposure of so-called "aggregation-prone motifs". Thus, in the various human blood group O-phenotypes, the fundamental *O*-glycan emptiness of the IgM molecule and its associated *N*- and *O*-glycan complementarity suggests the transient expression of ancestral A/B-like glycans that are "lost" over the course of germ cell maturation and/or cell renewal processes (Arend, 2016).

Consanguinity leads to self-incompatibility and a small population size

According to the previously proposed concept, a hypothetical population of continuously inbreeding blood group O(H) individuals may accelerate an evolutionary trend, characterized by a gradual reduction in silent blood group O-associated, A-specific GalNAc-formations, resulting in a complete loss of fertility (Arend, 2016). In general, the inbreeding of humans can obviously result in a complete loss of measurable ABO(H) expression; however, the loss of the functions of the FUT1 and FUT2 genes appears to reach a critical developmental and/or evolutionary point first. Indeed, the biological phenomenon of the Indian (Oh) *Bombay* type is primarily caused by a Tyr316Ter mutation in the coding region of FUT1, whereas other mutations underlie the Reunion H-weak (Fernandez-Mateos et al., 1998) and *Para-Bombay* types (Watkins, 1980; Wang et al., 1997; Dean, 2005). In contrast to the point mutations in the other glycosyltransferase genes, the ABO genes, the Lewis gene, and the secretor gene (Fernandez-Mateos et al., 1998; Kaneko et al., 1997; Wang et al., 1997), the H gene mutations are highly

variable. Most of these mutations apparently occur over the course of continued inbreeding and can be linked to specific consanguinities in certain families (Balgir, 2007). Reduced fertility leads to lower birth rates, which subsequently result in a small population size (Bittles and Black, 2010). After a decades-long debate on whether inbreeding in animals and humans affects fertility and survival, encompassing the *"Song Sparrows of Mandarte Island"* (Smith et al., 2006), California's *"Checkerspot Butterfly"* (Ehrlich and Hanski, 2004) and the *"Darwin/Wedgewood Dynasty"* (Berra et al., 2010), a recent analysis of the number of children per woman revealed a significantly adverse effect of the husband's inbreeding coefficient on family size and reproductive period duration (Álvarez et al., 2014). Thus, it appears appropriate that these authors stated that *"Darwin was right"* when, inspired by the male-linked inbreeding depression in his own family, assumed that reduced fertility in animals and plants predominantly emerges from inbreeding. Indeed, in higher-order plants, such as the flowers *Vicia villosa* (Hamadi et al., 2012) and *Vicia cracca* (Eliášová et al., 2014), self-fertilization creates self-incompatibility, which is considered to be the strongest genetic barrier to subsequent reproduction (Dixit & Nasrallah, 2001). The link between inbreeding depression and reduced reproductive performance has also been established by several observations in humans (Ober et al., 1999; Charlesworth and Willis, 2009; Inhorn et al., 2009; Paige, 2010). Not surprisingly, the prevalence of various *Bombay* types decreases markedly with urbanization, which reduces the rate of consanguineous marriages (Mallick et al., 2015).

Phenotype protects reproduction based on α1,2 fucosylation

According to ongoing debates on the effects of inbreeding depression on female and male gamete performances in plants and animals (Willis, 1999; Buchalski et al., 2014; Taylor et al., 2007; Kelly, 2003), reproduction among related individuals predominantly concerns the male gamete in diploid organisms (Opatová et al., 2016; Weeks et al., 2009), although a theory predicts that polyandrous murine females can avoid inbreeding by exploiting paternity-biasing mechanisms that enable differential sperm "use" (Firman and Simmons, 2015). In this context, it is intriguing that in the female mouse, fucosylation genes encoded from the polymorphic FX locus, which has an unknown epistatic/functional relation with the H and/or Se genes, determines neurobiological processes, such as sexual receptivity; for example, the lack of expression of the fucose mutarotase enzyme (FucM) in female FucM knock-out mice results in drastically reduced receptivity (Park et al., 2010). These authors explained the impact of

fucosylation on the complex endocrinology involved in the development of the male and female brain and how animal reproduction may depend on neurobiological processes. In this regard, the role of the human fucose mutarotase(s) awaits elucidation, although the fucose molecule obviously plays a greater role in the complex physiology of reproduction, and dominates the immunobiology of sperm-egg recognition and species/phenotype-intrinsic tissue maturation. This critical stage of growth involves the first steps of protein glycosylation in metazoan eukaryotes, in which the trans-species, phenotype-independent A-like mucin-type GalNAc-glycosylation of *O*-unprotected serine and/or threonine residues of progenitor cells is mediated by several as yet genetically undefined, *O*-GalNAc transferases (Tenno et al., 2007; Schjoldager and Clausen, 2012; Brockhausen et al., 2009). However, because the timing between human-specific α1,2 fucosylations and any other developmental and/or phenotype-related glycosylations remains unknown, an enzymatic cascade cannot be constructed, although again, the *Bombay* type development may indicate that these fucosylations must occur in early ontogeny.

In the human blood groups A and B, the intracellular and membrane-bound form of somatic glycotransferases, which innate the complex construction of the human ABO(H) histo (blood group) phenotypes in the trans cisternae and vesicles of the Golgi apparatus, are associated with soluble enzyme forms that exhibit identical specificities in the plasma. Consequently, the finding that a soluble GalNAc transferase purified from the plasma of a blood group A_1 donor displayed blood group A_1 conversion of the fucosylated blood group O(H) on the red cell surface *in vitro* (Nagai et al., 1978), indicates that this transferase activity may also affect the neonatal amino acid sequences of the innate IgM. In a normal human population, the developmentally timed expression of hydrophilic amino acids (Davis et al., 1989; Clark et al., 2006) could provide the clearance of anti-A auto-reactivity through germline-specific serine residues on the neonatal IgM (Willis et al., 2013), while somatically converted immunoglobulins are thought to undergo cellular internalization through a complex pathway, especially after *O*-glycosylation (Gill et al., 2011). The human-intrinsic A- and B-allelic GalNAc and D-Gal transferases, which occur in certain O(h) *Bombay* type individuals (Mulet et al. 1977), may not clear non-fucosylated isoagglutinin reactivities (Fig. 3), while blood group O(H) formation by α1,2 fucosylation involves further glycosylation options and appears to function as an evolutionary, metabolic crossover point between the trans-species Tn/T glycosylations and phenotypic, A/B-allelic glycosylation (Arend 2018) (Fig. 4), and may

provide the immunological balance between physiological, regulatory anti-self-reactive and aberrant glycosylation processes, and explain the normal reproductive performances of blood group O(H) individuals. Thus, in a revision of the previous concept (Arend, 2012; 2014; 2016), the classic A allele may merely contribute to protection via the addition of extra carbohydrate moieties. Furthermore, although a pronounced expression of EGF on blood group A_1 red cell membranes (Engelmann et al. 1992) suggests accelerated cell growth in general, a specific role of the ABO locus on chromosome 9 may even be questioned with regard to fertility.

Conclusions

The immunology of reproduction is a complex process, which ranges from GC maturation to implantation of the fertilized egg and ontogeny of the growing embryo in the female, while GC maturation obviously utilizes immunological mechanisms that are distinct from the protection of the ovulated egg and its transport. The "bulky" GalNAc-molecule (Yamamoto et al. 2001), which possesses undefined biophysical properties, clearly dominates many physiological functions based upon the various terminal and non-terminal *N*- or *O*-linked positions on polypeptides, glycoproteins and glycosphingolipids, whereby all lectin and antibody reactions primarily appear as A-like structures. Again, it needs to be emphasized that the non-somatic fleetingly expressed, serologically A-like trans-species functional *O*-GalNAc glycosylation, which is identical with the ontogenetic GalNAcα1-*O*-Ser/Thr-R epitope, also referred to as the Tn antigen and typically accumulates in cancerous tissues by various derivatives on aberrant protein backbones, is independent of any phenotype and was likely revealed by the early ovariectomy of the C57/BL/10 mouse (Arend 1977; 1977a). This intermediate epitope must be differentiated from somatic, human blood group A-specific, primarily membrane bound *N*-linked glycosylations, encoded by the A allele after formation of the zygote. Together with the B allele, the A-allele accomplishes human ABO(H) blood group completeness *per se* but obviously contributes little with regard to fertility. In contrast, the extremely small *Bombay* type population and the potency of fucosyl-depleted immunoglobulins promoting anti-self-reactivity suggest the developmental and/or evolutionary dominance of the H-, Se and/or Lewis genes. These genes, which are located on chromosome 19, and balancing the site-specific positions of fucosyl residues and protection of non-

somatic/ontogenic glycosylation during GC maturation and cell renewal processes, may call in question the primary role of the ABO locus on chromosome 9 with regard to fertility. Moreover, the central immunological position of the human histo (blood) group O(H) remains evident in its evolutionary metabolic cross-point between the regulation of trans-species glycosylations on one side and its role as the structural basis of species intrinsic glycosylation or phenotype formation on the other side (Arend, 2018). This extensive molecular biological task is evident in comprehensive presentation of both polyreactive nonimmune IgM and adaptive IgM/IgG antibodies against all of the mature A and B glycans and their cross-reactive developmental structures Tn and T, as illustrated in Figure 4. Not surprisingly, the blood group O(H) phenotype represents the most common blood type worldwide (Dean, 2005), and the extremely rare native O(h) *Bombay* type, which is characterized by the lack of the FUT1- and FUT2-encoding genes, may be attributed to a molecular mechanism of mutational meltdown, which leads to immunological self-incompatibility in the male genome, potentially determining the population size. Furthermore, the O(h) *Bombay* type most likely represents a species-intrinsic molecular biological phenomenon that is exclusively restricted to humans. Although humans and mice may lay the foundation of genome zoology (Emes et al. 2003) and have many properties in common, mice with targeted deletions of the FUT1 or FUT2 α (1,2) fucosyltransferase loci have been reported to display normal fertility (Domino et al. 2001). This fundamental difference in fucosylation and its encoding between rodents and primates awaits confirmation, whereas the difference between murine and human adaptive and innate immunity (Mestas and Hughes 2004) appears to have been established by pioneering experiments in animals and clinical trials using human volunteers, as reviewed by Khanna and Khanna (2011). In addition, the murine-specific equivalent of the human AB-glycotransferase (Yamamoto et al. 2001) and the difference in the carbohydrate-determined subspecificities between murine and

human innate IgM (Larkin and Porter, 2005) render the mouse an unsuitable model of the ABO(H)-phenotype discordance in primates. Furthermore, primates use different pathways for critical fucosylation events (Dupuy et al. 2002), and the α1,2-fucosyltransferase genes may exclusively accomplish the expression of ABO(H) epitopes and phenotype protection in humans, as suggested by the relationship between an intronic Alu-Y-element and red blood cells (Apoil et al. 2000). Finally, the aberrant fucosylation and infertility-related small population size of the *Bombay* type suggest that a human-specific fucosylation, which initiates the formation of the ABO/H) phenotypes (Arend, 2016; 2017), involves an identical fucosylation of immunoglobulins and contributes to the maintenance of species and phenotype diversity via a mechanism of immunologically-controlled anti-self-reactivity during reproduction. In fact, it is this molecular biological and immunogenetical background, which potentially contributes to the reduction in the overall risk of developing cancer of the blood group O(H) when compared with non-O(H) blood group phenotype carriers (Zhang et al., 2014; Hsiao et al., 2015), while it clearly protects the individuals of this group from life-threatening infections with evolutionary selective malaria strains (Arend 2018).

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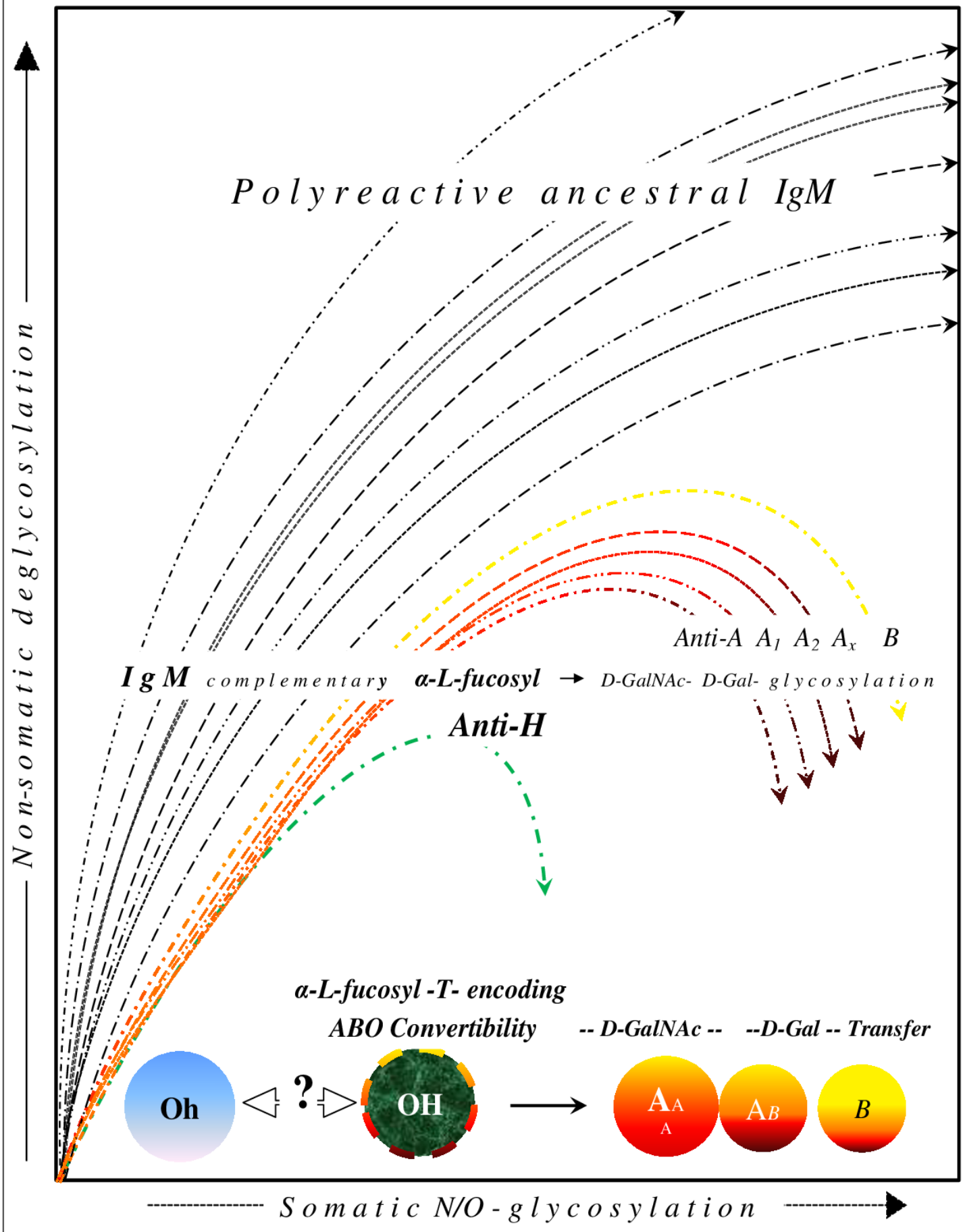
Polyreactive ancestral IgM
Non-somatic deglycosylation
Anti-A A1 A2 Ax B
IgM complementary α-L-fucosyl → D-GalNAc- D-Gal- glycosylation
Anti-H
α-L-fucosyl -T- encoding
ABO Convertibility
-- D-GalNAc --
--D-Gal -- Transfer
Oh
?
OH
AA
A
AB
B
Somatic N/O-glycosylation

Figure 1. In normal conditions, the simultaneous appearance of an ABO(H)-phenotype and its corresponding antibody is reduced or excluded by the identical, phenotype-specific glycosylation of complementary domains on the cell surfaces and plasma proteins, which involves the expression and/or secretion of the ancestral, non-differentiating IgM. Meanwhile the non-glycosylated, still polyreactive IgM remains available to the organism for the internal and external defences. The ABO(H) phenotype and innate isoagglutinin specificities arise from "glycosidic exclusion" (Arend 2016). This scheme demonstrates how fucosylation provides ABO(H) "blood group completeness". In metazoans, the germline-encoded, polyreactive ancestral IgM emerges as a glycan-depleted molecule from non-somatic germ cell maturation and developmental cell renewal or growth processes (Arend, 2017). Although the specific timing between the encoding of the fucosyl-, GalNAc-, and D-Gal transferases remains an open question, fucosylation is known to occur on both the cell surface and plasma proteins, followed by the transfer of GalNAc and D-Gal to the cell surfaces and the complementary domains of the differentiating IgM. This figure was constructed by Arend (2016).

Growth and the degree of α1,2 fucosylation/GalNAc glysolation of plasma proteins occur inversely proportional to the levels of natural complementary antibodies

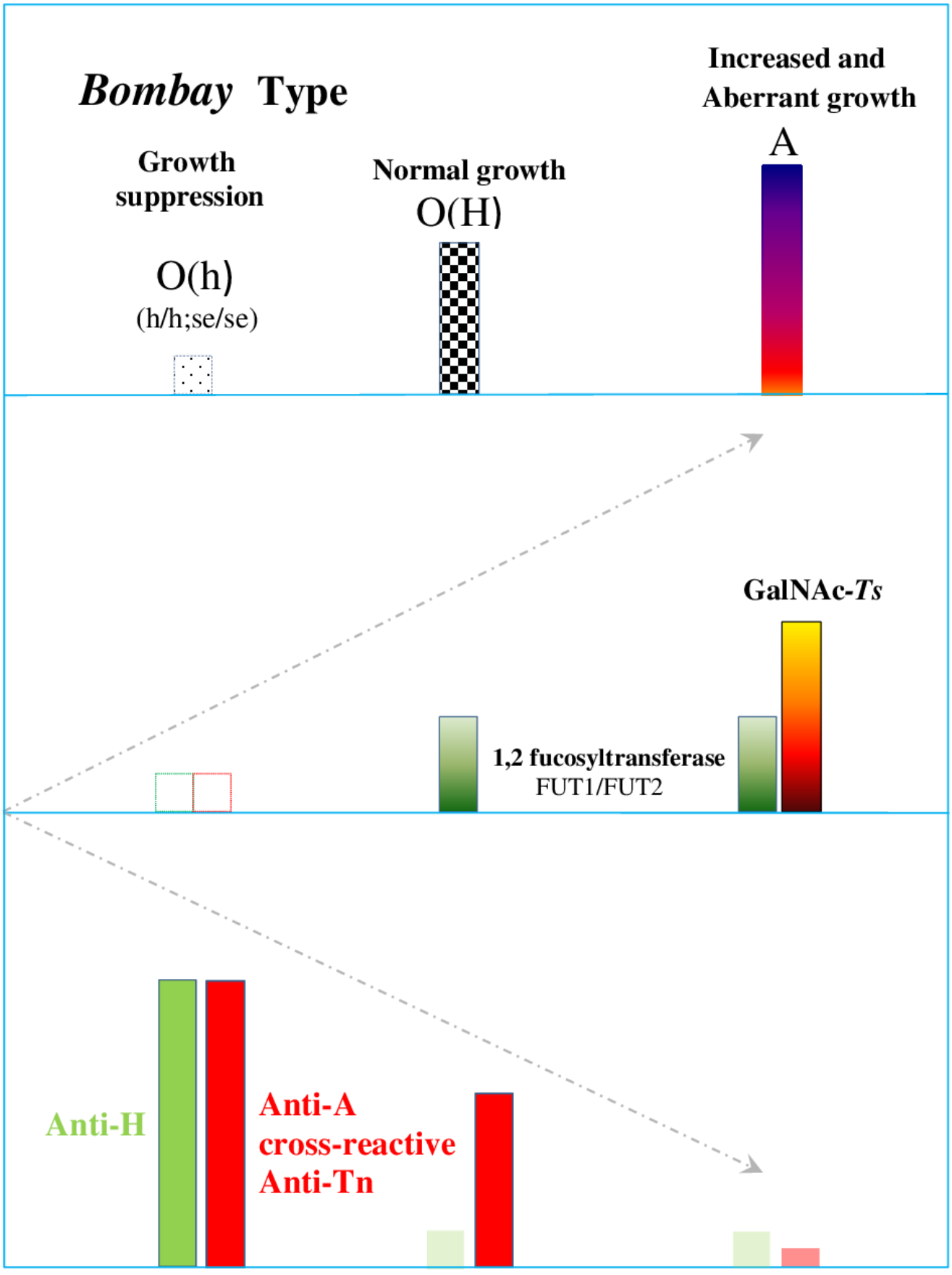

Figure 2. ABO(H) phenotype formation occurs on both the cell surfaces and plasma proteins. In the O(h) *Bombay* non-phenotype, which is not protected by clonal selection with regard to ABH antigenic structures, the process of embryogenic stem cell-to-germ cell transformation, dominated by metazoan (A-like) GalNAc α1-*O*-Ser/Thr-protein glycosylations and deglycosylations, is exposed to extremely increased reactivities of adaptive and non-immune anti-glycan immunoglobulins, involving a complex, unusual anti-H reactivity and pronounced anti-A/Tn cross-reactivity. In the classic blood group O(H), this exposure is modulated by human-specific α1,2 L-fucosylation, providing the basis for the ABO(H) phenotype formation, and converting an aggressive (auto) immunization into normal, physiological growth regulation. This developmental balance gets lost by blood group A-phenotypic GalNAc glycosylation. While promoting species phenotype diversity, this glycosylation is assumed to stimulate cell growth (Engelmann et al. 1992) but simultaneously affects innate immunity by reducing or removing the growth-regulating function of germline-encoded anti-A/Tn IgM cross-reactivity. Therefore, this glycosylation might significantly be associated with the formation of "aberrant" structures and an increased overall risk of developing cancer when compared with that, dominating blood group O(H) development.

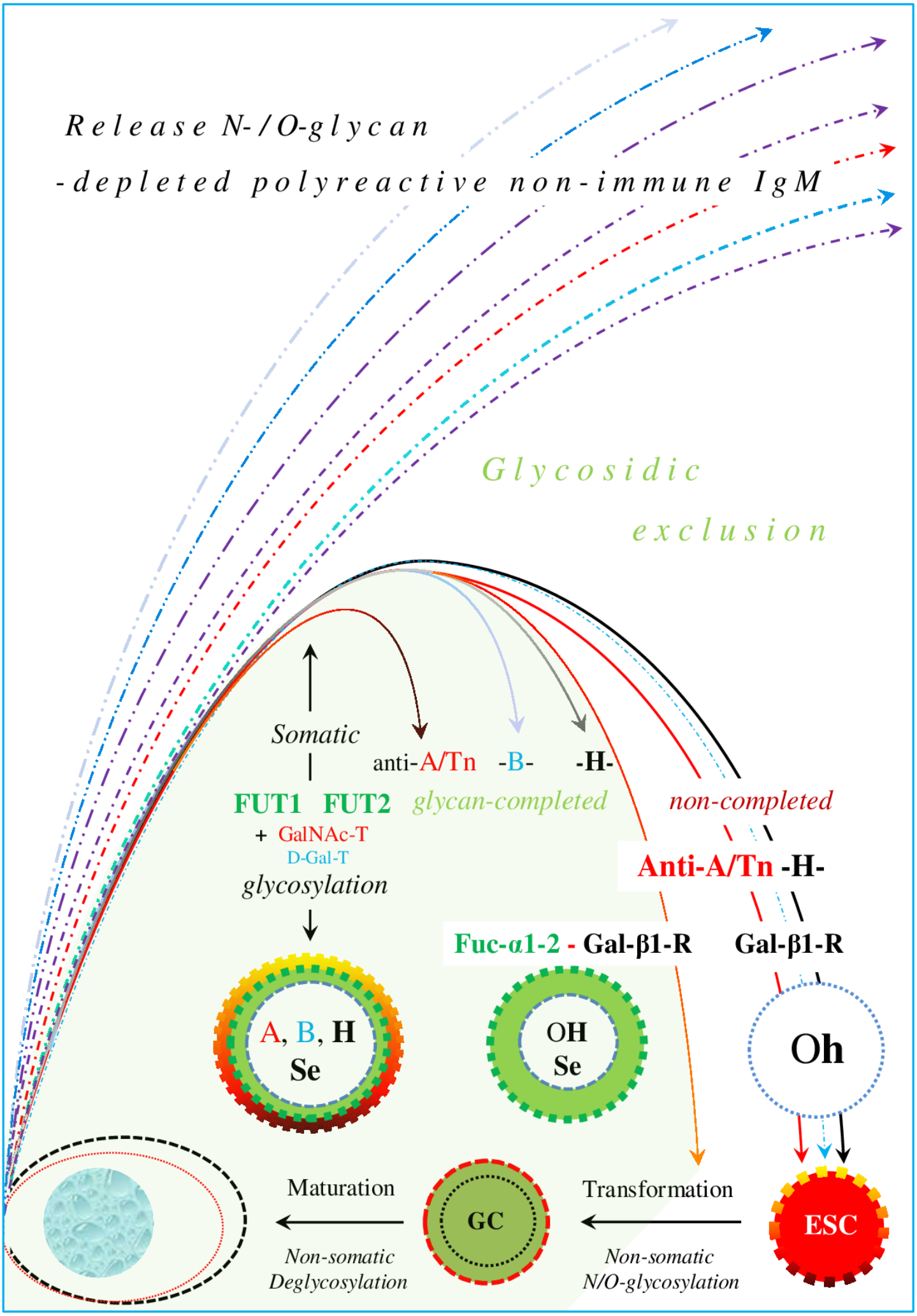
Release N-/O-glycan
-depleted polyreactive non-immune IgM
Glycosidic
exclusion
Somatic
anti-A/Tn
-B-
-H-
FUT1 FUT2
+ GalNAc-T
D-Gal-T
glycosylation
glycan-completed
non-completed
Anti-A/Tn -H-
Fuc-α1-2 - Gal-β1-R
Gal-β1-R
A, B, H
Se
OH
Se
Oh
Maturation
Non-somatic
Deglycosylation
GC
Transformation
Non-somatic
N/O-glycosylation
ESC

Figure 3. Glycosidic exclusion and/or accommodation do not protect the *Bombay* type. The phenotypes and non-immune isoagglutinin specificities of the human histo (blood) group ABO(H) system are hypothesized to arise together in a molecularly connected synthetic pathway, which may be called glycosidic exclusion (Arend 2016). This pathway refers to a physiological principle in which the simultaneous appearance of a phenotype and non-immune (IgM) isoagglutinins is protected from anti-self-reactivity by the identical glycosylation of complementary domains on cell surfaces and plasma proteins under normal conditions, resulting in the creation and cellular internalization (Gill et al. 2011) of glycan-completed IgM (Arend 2016) and/or immunoglobulins in general. This mechanism is thought to be primarily be based on somatic fucosylation. While the plasma of the normal blood group O(H) is associated with a mixture of adaptive and non-immune anti-A/Tn and B-reactive immunoglobulins, the levels of these immunoglobulins are strongly increased in the non- or poorly fucosylated O(h) or *Bombay* type plasma, which additionally exhibits a complex anti-H reactivity and pronounced non-immune anti-A/Tn cross-reactivity, highly suggesting the induction of a complex, complement-mediated cytotoxicity against the stem cell to germ cell transformation.

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

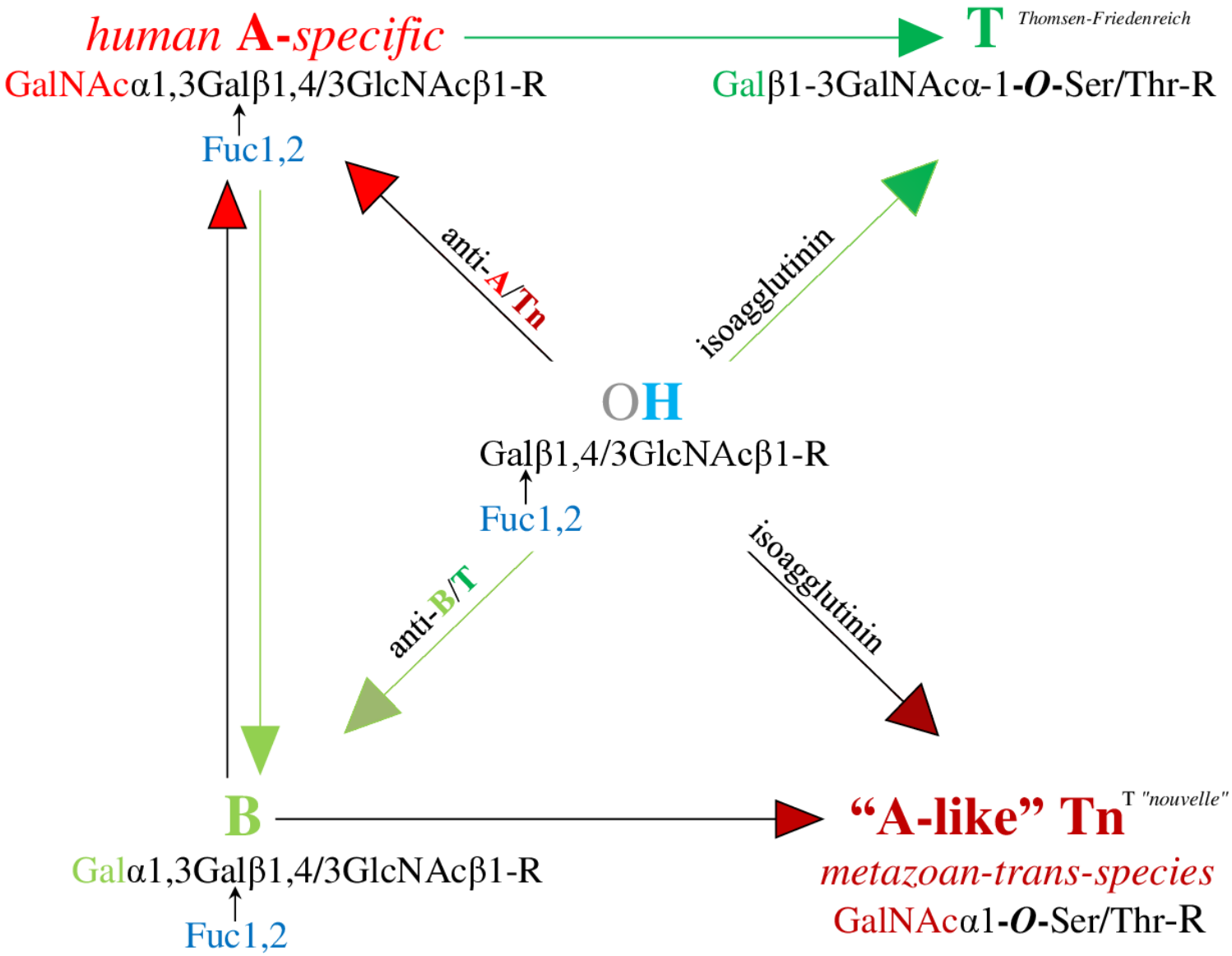

Figure 4. The central immunological position of the blood group O(H) is evident in its comprehensive production of "natural" antibodies against all of the mature A and B glycans and their cross-reactive developmental structures Tn and T. The human A-specific (A-allelic) glycosylation and the trans-species "A-like" Tn formation are developmentally connected via the formation of cross-reactive anti-A/Tn isoagglutinin. According to Hofmann et al. (2014), 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 the anti-Tn antibody, which does not react with blood group B red cells or T glycoconjugates. By contrast, the anti-B antibodies of blood group A sera and of blood group O(H) sera bind to B and T glycoconjugates but not to A or Tn glycoconjugates. This selective cross-reactivity of isoagglutinins with Tn and T antigens has been explained by the authors through the phenotype-specific terminal moieties; the terminal N-acetylgalactosamine is shared by A and Tn antigens, and the terminal galactose is, although with a different configuration, shared by B and T antigen. This figure was constructed by Arend, P. (2017).