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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/Wedgwood 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.

1. 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).

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

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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, 2007).

2. 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, 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 (2005a, 2005b). 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 °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 and 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 and 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. (1981), ADCC may not to be mediated by IgM alone, and traces of IgG are always needed; additionally, 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 and 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). While promoting species diversity, human blood group A-determining glycosylation assumingly dominates the growth factor activity (Engelmann et al., 1992), but when stimulating epithelial cell turnover simultaneously affects innate immunity by reducing or removing the growth-regulation function of germline-encoded antiA/Tn cross-reactivity (Arend, 2017), and thus is associated with the formation of aberrant structures and an increased risk of developing cancer. 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.